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American Journal of Veterinary Research

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The Behavior of Brucella Abortus Vaccine in Various Excipients*

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INCREASING interest has been displayed by many investigators during the past ten years in the possibility of retarded antigen absorption being a means of inducing greater immunological response. The encouraging results obtained in many of these earlier studies have been responsible for much of the current experimental work being conducted along these lines.

In general, those methods by which retarded absorption can best be accomplished are based on the principles of nonabsorbability of certain bland oils and the tendency for encapsulation that results from the presence of mild irritants within body tissues. In the case of nonabsorbable materials, suspended particles appear to be held at the site of injection by a protective coating of oil, while a similar localizing action is secured by the connective tissue hyperplasia resulting from mild irritations.

The practical application of these principles is seen in the extensive use of anthrax vaccines suspended in physiological saline containing small percentages of saponin. Mazzucchi[1] first reported on the use of saponin in connection with anthrax vaccines in 1929. The presence of this material has been demonstrated as having no appreciable effect on the virulence of the cultures, but showing a tendency to induce localization of the organisms at the site of injection. They may remain viable at this point for a period of from one to two weeks. Their extension into the surrounding tissues is prevented by a definite wall of demarcation and eventual destruction of the organisms takes place as a result of phagocytosis and plasmolysis.

Ramon, Lemetayer, and Richou[2] in 1937 reported on the antigenic value of mixtures of cholesterol in liquid petrolatum and lanolin in olive oil in connection with *Corynebacterium diphtheriae* and *Clostridium tetani* anatoxins. They found these suspensions to be more efficient antigens than the toxoids alone. This enhanced antigenic value has been explained on the theory that when a mixture of anatoxin and lipoid substances is injected, small amounts are released into the body gradually, thus stimulating a higher degree of antitoxin formation.

Further reports on the use of lanolin were submitted by Maccolini[3] in 1938. In these investigations, the author was able to demonstrate a definitely enhanced immunity obtained in mice with *Salmonella aertrycke* and *Eberthella typhosus* antigens containing 25 per cent lanolin.

Velu and Zottner[4] in 1938 reported on the results of their investigations dealing with *Brucella abortus* vaccines incorporated in lanolin and liquid petrolatum. In their opinion, *Br. abortus* cultures suspended in 50 per cent lanolin and liquid petrolatum are rapidly destroyed but continue to display a high antigenic value as well as a complete harmlessness for man and animals.

Numerous field experiments in connection with vaccination against Bang's disease are

*From the Animal Disease Station, Bureau of Animal Industry, U. S. Department of Agriculture.

[1]Mazzucchi, M.: Soc. Internaz. d. Microbiol., iii (1929), p. 193.

[2]Ramon, G., Lemetayer, E., and Richou, R.: Rev. Immunol., iii (1937), pp. 202-218.

[3]Maccolini, Roberto: Bull. Soc. Ital. Biol. Sperim., xiii (1938), pp. 1079-1081.

[4]Velu, H., and Zottner, G.: Bull. Acad. Vét. Fr., xi (1938), pp. 42-51.

discussed in a report by Dubois[5] in 1938 covering the vaccination of ewes, goats, and cattle in various parts of France. The vaccine employed consisted of viable *Br. suis* combined with lycopodium powder in liquid petrolatum and lanolin base. As a result of vaccination of 23,000 cattle, sheep, and goats, it is stated that the number of abortions and cases of sterility were markedly reduced. Dubois is of the opinion that such a vaccine may be safely administered during gestation and that a single injection will confer a high degree of immunity. An inadequate number of bacteriological examinations failed to reveal the presence of the organisms in the blood, urine, and milk of a limited number of vaccinated animals. The absence of controls in a field experiment of this nature obviously leaves the results obtained open to justifiable criticism.

Studies conducted by Cordier[6] and reported in 1938 were based on the use of vaccines prepared with suis and bovine strains of Brucella, suspended in various mixtures of lanolin in liquid petrolatum as well as lanolin in olive oil. The author's observations on the results of vaccination experiments conducted on sheep, goats, guinea pigs, and a limited number of cattle present further evidence of the tendency for such vaccines to localize promptly at the site of injection. It is stated that repeated inoculations of sheep invariably failed to produce any carriers of the infection. The persistence of specific agglutinins and allergy in the vaccinated animals was shown to be of longer duration in the case of avirulent cultures suspended in oily mixtures than was true when broth cultures of the same strain were used.

In connection with the control of Bang's disease, the danger of permanently establishing the infection in animals vaccinated with viable cultures has been largely obviated through the use of established strains of low virulence and confining their use to calves of from 4 to 8 months of age. On the basis of results obtained in carefully controlled vaccination experiments conducted at the Animal Disease Station, it is believed that under the above procedure the danger of permanent infection being established as a result of vaccination is so remote as to be negligible.

While the results obtained under this procedure have been highly encouraging, the scope of vaccination could be materially broadened through the development of an agent capable of safely inducing a high degree of permanent resistance in animals of all ages.

In view of the foregoing and for the purpose of securing additional data relative to the influence of various excipients on antigenic activity, the following studies have been undertaken.

EXPERIMENTAL PROCEDURE

These investigations have had as their ultimate object the determination of the relative merits of *Br. abortus* vaccines suspended in various excipients, primarily from the standpoint of induced immunological response. This has necessitated considerable preliminary investigation in order that an otherwise extensive field of possible agents could be narrowed down to a selected few of sufficient promise to warrant further study.

The materials utilized in these investigations have been confined to those having recognized physical characteristics of a desirable nature. Such factors as absorbability, irritant action, and miscibility have been taken into consideration in each case. The percentage combinations of the various agents employed were arrived at on the basis of physical as well as physiological properties.

The following materials were selected for preliminary study:

2% cholesterol in oil of sweet almond
4% cholesterol in liquid petrolatum
6% cholesterol in liquid petrolatum
2% cholesterol in oil of sweet almond
4% cholesterol in oil of sweet almond
6% cholesterol in oil of sweet almond
1% saponin in physiological saline
2% saponin in physiological saline
10% lanolin in liquid petrolatum
25% lanolin in liquid petrolatum
50% lanolin in liquid petrolatum
100% oil in sweet almond
100% liquid petrolatum

[5]Dubois, C.: Bull. Acad. Vét. Fr., xi (1938), pp. 383-404.
[6]Cordier, G.: Rev. de Méd. Vét., xc (1938), pp. 481-503.

Relative values were determined on the basis of direct comparison with 0.85 per cent saline.

In preparing the excipients for incorporation with the organisms, all materials were measured and combined in the desired proportions, prior to sterilization at 120° C. for 30 minutes.

Br. abortus (bovine) strain 19, utilized throughout the experiment, is one of low virulence but not avirulent and has been extensively employed in the preparation of saline suspended vaccine.

The bacterial suspensions were prepared in quantity from 48-hour growths, obtained from potato agar in 32-oz. Blake production bottles. The cell content of this material, in terms of percentage, was ascertained by means of the Hopkins vaccine tube. Based on this determination, it was possible to approximate within a relatively close range the actual number of organisms added to a given excipient. By means of direct microscopic count, it has been demonstrated that a suspension of *Br. abortus* strain 19, having a cell content of 0.5 per cent, will contain approximately 9 x 10^9 organisms per cubic centimeter. Through the addition of definite quantities of the excipient to calculated amounts of sedimented cells, bacterial suspensions of desired concentrations were obtained. Direct microscopic as well as cultural viability counts were conducted on all suspensions for verification of cell content.

IN VITRO DETERMINATIONS

The *in vitro* studies were carried out in an effort to determine the effect of various excipients on the viability of *Br. abortus* held under different ranges of temperature. Bacterial suspensions prepared in triplicate, were adjusted in such a manner as to insure a uniform cell content of approximately 5 x 10^9 organisms per cubic centimeter. Identical suspensions prepared in each of the excipients were held at a) room temperature, 20 to 28° C.; b) incubation temperature, 37.5° C.; and c) refrigerator temperature, ±8° C. Viability counts were made at the time of preparation for comparison with those secured at subsequent intervals. Routine determinations on the number of viable organisms present in each suspension were conducted at regular intervals of seven days. The procedure employed in these counts consisted of plating each suspension, in countable dilutions, on serum potato agar. All plates were examined and the number of colonies determined at the end of a 72-hour incubation period, the results being recorded in terms of viable organisms per cubic centimeter. In this connection, the use of a wide-field dissecting microscope was found to be of value from the standpoint of speed as well as accuracy.

RESULTS

The effect of various excipients on the viability of *Br. abortus*, under different temperature ranges, is recorded in tables I, II, and III.

It is apparent that a pronounced difference exists among many of these agents in respect to their bactericidal action on *Br. abortus*. While this action is shown to be markedly accentuated when the mixtures are held at incubation temperatures, the same effects, to a less degree, are demonstrable in the same mixtures held at lower temperatures. Direct correlation relative to the bactericidal properties exhibited by a given excipient under various temperature ranges is apparent; existing differences being manifest in degree rather than in effect.

The remarkable germicidal properties of lanolin and liquid petrolatum mixtures, *in vitro*, are apparent in the rapid sterilization of viable *Br. abortus* suspensions that occurs under all temperatures studied. This bactericidal action has, in all cases, closely paralleled the percentages of lanolin employed. These results are somewhat in line with those secured by Velu and Zottner,[4] in which the mixture of 50 per cent lanolin in liquid petrolatum completely sterilized *Br. abortus* suspensions within eight hours when held at 20 to 37° C. and 48 hours at 3° C.

Oil of sweet almond, containing small percentages of cholesterol, exhibits a similar action on the viability of *Br. abortus*, but to a less degree.

TABLE I—Effect of various excipients on Br. abortus in vitro. Incubator temperature—37.5° C.

Excipient	Determinations at Designated Intervals							
	Days							
	0	7	14	21	28	35	42	49
	Viable Br. Abortus per cc.							
50% lanolin liquid petrolatum	$800x10^6$	0	0	0				
25% lanolin liquid petrolatum	$950x10^6$	0	0	0				
10% lanolin liquid petrolatum	$1,100x10^6$	0	0	0				
2% cholesterol oil of sweet almond	$1,000x10^6$	0	0	0				
4% cholesterol oil of sweet almond	$950x10^6$	0	0	0				
6% cholesterol oil of sweet almond	$1,100x10^6$	0	0	0				
100% oil of sweet almond	$850x10^6$	$70x10^6$	$3.1x10^6$	0	0	0		
4% cholesterol liquid petrolatum	$850x10^6$	$210x10^6$	$34x10^6$	$3.1x10^6$	$1.1x10^6$	0	0	
6% cholesterol liquid petrolatum	$900x10^6$	$125x10^6$	$41x10^6$	$20x10^6$	$810x10^3$	0	0	
2% cholesterol liquid petrolatum	$980x10^6$	$140x10^6$	$48x10^6$	$2.8x10^6$	$990x10^3$	$4.5x10^3$	0	
100% liquid petrolatum	$990x10^6$	$240x10^6$	$7.2x10^6$	$1.8x10^6$	$670x10^3$	$48x10^3$	908	0
2% saponin	$1,200x10^6$	$1,090x10^6$	$150x10^6$	$41x10^6$	$21x10^6$	$5.5x10^6$	$3.7x10^6$	$500x10^3$
1% saponin	$1,380x10^6$	$910x10^6$	$250x10^6$	$28x10^6$	$22x10^6$	$11x10^6$	$5x10^6$	$950x10^3$
Saline	$1,500x10^6$	$150x10^6$	$55x10^6$	$18x10^6$	$8x10^6$	$3.2x10^6$	$910x10^3$	10^3

0 indicates negative culture results.

Complete absence of viability was demonstrated in the 2, 4, and 6 per cent cholesterol in oil of sweet almond, within seven days at 37.5° C.

Those excipients in which viability appears to be maintained, include saponin in 1 and 2 per cent concentrations, 100 per cent liquid petrolatum, 2 per cent cholesterol in liquid petrolatum, as well as physiological saline. In this connection, physiological saline appears to be surpassed only by 100 per cent liquid petrolatum when comparisons are made on the basis of *in vitro* determinations.

While the data are incomplete at the present time, particularly in regard to the duration of viability under refrigeration temperatures, it would appear that, except for a much retarded rate, the same general relationship between excipients and viability will exist under these conditions, as was observed at higher temperatures.

Animal Inoculations

In order to evaluate further the various excipients under investigation, experiments dealing with their action as vehicles for *Br. abortus* vaccines were undertaken. The preliminary studies in this connection have had as their object a comparison of the relative merits of these materials from the standpoint of 1) local and general reactions produced, 2) the duration of viability of the suspended organisms at the sites of injections, and 3) the immunological response induced in the vaccinated animal.

Group 1—Inoculated Cows

The original observations in this connection were made on a group of 13 mature cows, ranging in age from 3 to 4 years. The inoculum employed in this group of animals consisted of viable *Br. abortus* strain 19 suspended in each of the excipients selected for use in this part of the work. One control animal received similar injections of saline suspended vaccine. The bacterial content was standardized in such a manner as to insure the inoculation of approximately uniform numbers of organisms in all cases.

In order to assure adequate sites, as sources of material for bacteriological examinations, each animal, with the exception of the two saponin-injected cows,

received subcutaneous injections of 2, 2, 4, and 6 cc., respectively, at four sites. Due to the irritant action connected with the subcutaneous administration of saponin, the amounts injected were reduced to half that employed in the other excipients.

The injections were made under the skin in shaved and disinfected areas approximately 10 cm. square, situated anteriorly and posteriorly to the shoulder on each side. The vaccine employed in individual animals was confined to multiple injections incorporating the same excipient. In all cases the number of organisms injected remained constant—40 x 10^9—regardless of the amount of suspending medium.

Daily observations relative to the size and character of local reactions were made on each animal during the first ten days. Subsequent examinations, as well as removal of material from vaccinal sites for bacteriological determinations, were conducted at weekly intervals. The material secured for viability culture was, in most cases, obtained by aspiration with sterile 2-cc. syringes and 16-gauge needles. In those instances where aspiration was impossible, a tissue biopsy was performed at the injection site. Adequate precautions were exercised to preclude the possibility of this material becoming contaminated from external sources. Measured amounts of the aspirated material were plated on serum potato agar in such dilutions as to insure determinations in approximate number of viable organisms per cubic centimeter.

RESULTS

General Reactions.—The temperatures of the cattle, taken prior to the administration of vaccines, and daily for a period of seven days thereafter, failed to reveal significant differences in the thermic reactions observed in any of the animals. In general, the temperatures showed a rather abrupt rise of approximately 3° F. during the first twelve hours. These uniformly subsided, until at the end of 72 hours following inoculation, practically all had returned to normal.

Local Reactions.—Observations relative to the size and character of local reactions were made at regular intervals in order

TABLE II—Effect of various excipients on Br. abortus in vitro. Refrigerator temperature— ± 8° C.

EXCIPIENT	DETERMINATIONS AT DESIGNATED INTERVALS							
	DAYS							
	0	7	14	21	28	35	42	49
	VIABLE BR. ABORTUS PER CC.							
50% lanolin liquid petrolatum	800x10^6	78x10^6	3x10^6	400x10^3	80	0	0	0
25% lanolin liquid petrolatum	950x10^6	140x10^6	78x10^6	40x10^6	8x10^6	1.8x10^6	400x10^3	350x10^3
10% lanolin liquid petrolatum	1,100x10^6	260x10^6	210x10^6	160x10^6	80x10^6	30x10^6	800x10^3	780x10^3
2% cholesterol liquid petrolatum	980x10^6	562x10^6	250x10^6	180x10^6	80x10^6	24x10^6	4.8x10^6	3.6x10^6
4% cholesterol liquid petrolatum	850x10^6	320x10^6	140x10^6	100x10^6	30x10^6	12x10^6	1.2x10^6	950x10^3
6% cholesterol liquid petrolatum	900x10^6	280x10^6	125x10^6	80x10^6	28x10^6	6.8x10^6	1.1x10^6	750x10^3
2% cholesterol oil of sweet almond	1,000x10^6	562x10^6	245x10^6	80x10^6	42x10^6	15x10^6	268x10^3	190x10^3
4% cholesterol oil of sweet almond	950x10^6	350x10^6	175x10^6	90x10^6	39x10^6	10.5x10^6	85x10^3	95x10^3
6% cholesterol oil of sweet almond	1,100x10^6	310x10^6	200x10^6	65x10^6	42x10^6	8.5x10^6	60x10^3	40x10^3
100% oil of sweet almond	850x10^6	700x10^6	250x10^6	182x10^6	35x10^6	4.5x10^6	24x10^3	20x10^3
100% liquid petrolatum	990x10^6	850x10^6	640x10^6	450x10^6	310x10^6	145x10^6	31x10^6	24x10^6
2% saponin	1,200x10^6	410x10^6	180x10^6	35x10^6	20x10^6	15x10^6	5x10^6	10^6
1% saponin	1,380x10^6	520x10^6	130x10^6	28x10^6	18x10^6	12x10^6	8x10^6	3.5x10^6
Saline	1,500x10^6	700x10^6	180x10^6	43x10^6	25x10^6	16x10^6	12x10^6	10x10^6

0 indicates negative culture results.

TABLE III—Effect of various excipients on Br. abortus in vitro. Room temperature—(22 to 28° C.)

Excipient	Determinations at Designated Intervals							
	Days							
	0	7	14	21	28	35	42	49
	Viable Br. Abortus per cc.							
50% lanolin liquid petrolatum	$800x10^6$	0	0	0				
25% lanolin liquid petrolatum	$950x10^6$	$24x10^6$	$800x10^3$	$17x10^3$	0	0	0	
10% lanolin liquid petrolatum	$1,100x10^6$	$55x10^6$	$2.5x10^6$	$600x10^3$	0	0	0	
4% cholesterol oil of sweet almond	$950x10^6$	$91x10^6$	$4.2x10^6$	$2x10^6$	$228x10^3$	$6.3x10^3$	0	
6% cholesterol oil of sweet almond	$1,100x10^6$	$83x10^6$	$3.8x10^6$	$3.1x10^6$	$200x10^3$	$8.1x10^3$	0	
2% cholesterol liquid petrolatum	$980x10^6$	$110x10^6$	$65x10^6$	$5x10^6$	$3.8x10^6$	$2.2x10^6$	$7.1x10^3$	970
4% cholesterol liquid petrolatum	$850x10^6$	$120x10^6$	$52x10^6$	$5.8x10^6$	$4.3x10^6$	$1.1x10^6$	$8.5x10^3$	450
6% cholesterol liquid petrolatum	$900x10^6$	$112x10^6$	$45x10^6$	$4.8x10^6$	$3x10^6$	$890x10^3$	$3.5x10^3$	10^3
2% cholesterol oil of sweet almond	$1,000x10^6$	$85x10^6$	$4.8x10^6$	$2.4x10^6$	$277x10^3$	$38x10^3$	870	0
100% oil of sweet almond	$850x10^6$	$580x10^6$	$180x10^6$	$28x10^6$	$6.5x10^6$	$1.5x10^6$	$24x10^3$	780
100% liquid petrolatum	$990x10^6$	$600x10^6$	$410x10^6$	$250x10^6$	$175x10^6$	$15.1x10^6$	$1.8x10^6$	$850x10^3$
2% saponin	$1,200x10^6$	$800x10^6$	$110x10^6$	$30x10^6$	$14x10^6$	$9x10^6$	$4.5x10^6$	$5x10^6$
1% saponin	$1,380x10^6$	$610x10^6$	$100x10^6$	$31x10^6$	$16x10^6$	$9.5x10^6$	$3.8x10^6$	$3x10^6$
Saline	$1,500x10^6$	$210x10^6$	$49x10^6$	$21x10^6$	$13x10^6$	$4.2x10^6$	$2.2x10^6$	$1.8x10^6$

0 indicates negative culture results.

that any marked variations associated with the injection of different excipients might be noted.

The results of these observations indicated certain minor variations in the size of reactions. However, anticipated significant differences never became apparent. The most pronounced variation observed was in the persistence, rather than the size of these processes.

A maximum size of 6 x 16 x 2.3 cm. was observed in cow No. 2008, inoculated with 2 per cent saponin suspended vaccine. The smallest reaction was noted in cow No. 2003 which received vaccine prepared in 10 per cent lanolin and liquid petrolatum. The reactions in this case never exceeded the recorded measurement of 5 x 6.5 x .6 cm.

In the majority of cases the reactions were most pronounced within a period from 96 to 144 hours following injection. Gradual recession of the processes extended over a variable period of time. As would be expected, resorption occurred most rapidly in the case of saline suspended vaccine, where complete disappearance of the local reaction was observed in most instances within 14 days. On the other hand, persistence of reactions was most pronounced in the case of 50 per cent lanolin in liquid petrolatum and 4 and 6 per cent cholesterol in liquid petrolatum. Resorption in these cases extended over a period of from 65 days, in the case of 4 and 6 per cent cholesterol and liquid petrolatum mixtures, to over 150 days in connection with 50 per cent lanolin-liquid petrolatum preparations.

The tendency for rapid localization, as indicated by the early presence of definitely circumscribed swellings, appeared to be quite marked in the case of 2, 4, and 6 per cent cholesterol in liquid petrolatum mixtures. While eventual circumscription was apparent to some extent in all injections, this process was in many instances considerably slower than was observed in the cholesterol in liquid petrolatum suspensions. A tendency for gravitation of the injected material was observed in those instances where delayed localization was

apparent. Those excipients displaying this tendency to the greatest degree included 1 and 2 per cent saponin, 100 per cent oil of sweet almond, and 100 per cent liquid petrolatum.

Persistence of Viable Br. abortus at Vaccinal Sites.—The results of bacteriological examinations, relative to the persistence of viable Brucella at sites of injections, are summarized in table IV. It will be observed that a rather wide range of variation exists in connection with the various excipients employed. This is markedly apparent in the case of cow No. 2013, which was injected with 100 per cent liquid petrolatum, and in cow No. 1999, which received vaccinal inoculations suspended in 2 per cent cholesterol in liquid petrolatum mixtures. In the case of cow No. 2013, it was possible to recover viable Brucella from inoculation sites, for a period of only 30 days, while under the same procedure, positive cultures have been obtained from cow No. 1999, 150 days following the original injection.

The results obtained with 100 per cent liquid petrolatum are quite interesting, in view of the fact that viable Brucella first disappeared from inoculation sites where this excipient was employed. These findings are in direct contrast to the results of *in vitro* determinations on this material held at 37.5° C. As indicated in table III, 100 per cent liquid petrolatum appeared to possess admirable properties for the preservation of viability. Furthermore, similar contrasting results are seen in the action of 50 per cent lanolin in liquid petrolatum. While mixtures of this material appeared to possess definite bactericidal properties for Brucella in suspensions held *in vitro* at 37.5° C., this action was much less pronounced in animal inoculation. This fact is clearly demonstrated in the case of cow No. 1988, where it was possible to recover the vaccinal organism 65 days after inoculation.

The recovery of viable Brucella from vaccinal sites 65 days after inoculation, as demonstrated in cow No. 1978, seems quite remarkable in view of the fact that physiological saline was employed as a vehicle for the vaccine with which this animal was inoculated. In this connection, Haring and

TABLE IV—Recovery of viable Br. abortus from vaccinal sites. Group I—Mature cows.

Animal No.	Excipient	Days Subsequent to Injection																		
		10	20	30	37	45	51	59	65	73	83	91	97	108	115	122	129	136	143	150
		Results																		
2013	100% liquid petrolatum	+++	+	+	—	—	—	—												
2011	100% oil of sweet almond	+++	+	—	+	—	—	—	—											
1997	6% cholesterol oil of sweet almond	++	C	+	+	—	—	—	—											
1977	2% cholesterol oil of sweet almond	+++	++	+	+	+	—	—	—	—										
2014	1% saponin	+++	++	++	+	+	—	+	—	—	—	—								
1978	Saline	+++	++	++	+	+	—	+	+	—	—	—	—							
1988	50% lanolin liquid petrolatum	+++	+++	++	+	C	+	+	+	—	—	—	—							
2008	2% saponin	++	++	++	+	+	+	+	+	+	—	—	—	—						
2003	25% lanolin liquid petrolatum	+++	++	C	+	C	+	—	—	+	—	—	—	—	—	+	—	—	—	
2012	4% cholesterol oil of sweet almond	++	+	C	+	—	—	—	+	—	+	—	—	—	—					
1980	4% cholesterol liquid petrolatum	+++	++	+	+	+	+	+	+	+	+	—	—	—	—					
1987	6% cholesterol liquid petrolatum	+++	+++	+++	+++	++	++	++	+++	+++	++	—	C	—	+	+	—	—	+	C
1999	2% cholesterol liquid petrolatum	+++	+++	+++	+++	++	++	+++	++	+++	+++	++	+	+	+	+	—	—	+	+

+++Over $30x10^3$ Br. abortus per cc.
++10^3 to ($30x10^3$) Br. abortus per cc.
+Less than 10^3 Br. abortus per cc.
—No Br. abortus isolated.
CContaminated.

TABLE V—Blood agglutination titres of inoculated animals. Group 2—Yearling steers.

Animal No.	Excipient	Pre-Injection Titre	Days Subsequent to Injections					
			11	23	33	53	78	85
		Maximum Dilution in Which Agglutination Was Complete						
*2,153	2% cholesterol liquid petrolatum	Neg. 1:25	800	200	400	25	25	25
*2,244	2% cholesterol liquid petrolatum	Neg. 1:25	100	200	100	50	25	25
2,245	2% cholesterol liquid petrolatum	Neg. 1:25	100	200	100	400	100	100
2,246	2% cholesterol liquid petrolatum	Neg. 1:25	1,600	800	400	100	50	50
2,281	2% cholesterol liquid petrolatum	Neg. 1:25	1,600	800	800	400	400	200
2,247	2% cholesterol liquid petrolatum	Neg. 1:25	800	400	400	100	25	50
*2,239	1% saponin	Neg. 1:25	1,600	1,600	800	200	50	50
*2,209	1% saponin	Neg. 1:25	400	800	400	50	50	25
2,240	1% saponin	Neg. 1:25	400	400	200	400	50	100
2,241	1% saponin	Neg. 1:25	1,600	1,600	800	100	100	100
2,242	1% saponin	Pos. 1:25	100	200	200	100	50	50
2,243	1% saponin	Neg. 1:25	3,200	1,600	800	400	200	100
*2,248	Saline	Neg. 1:25	100	100	100	—	—	—
2,249	Saline	Neg. 1:25	3,200	800	400	100	50	100
2,250	Saline	Neg. 1:25	3,200	1,600	1,600	200	200	200
2,251	Saline	Neg. 1:25	50	400	400	100	25	50
2,252	Saline	Neg. 1:25	6,400	3,200	800	100	50	50
2,253	Saline	Neg. 1:25	3,200	3,200	1,600	200	200	200

*These animals each received single injections of 40×10^9 organisms.

Traum[7] have reported the isolation of strain 19 from subcutaneous tissues, near the point of inoculation, 24 days following a saline injection of 200×10^9 organisms.

Group 2—Inoculated Steers

In view of the results of the preceding experiment, it appeared advisable to investigate further those excipients possessing sufficient merit to justify additional study. The agents selected for this purpose included 1 per cent saponin and 2 per cent cholesterol in liquid petrolatum. The procedure was outlined in such a manner as to insure a careful comparison of these materials with physiological saline when employed as vehicles for *Br. abortus* vaccines.

For this work, 18 yearling steers were inoculated with *Br. abortus* suspensions in saline, 1 per cent saponin and 2 per cent cholesterol in liquid petrolatum. These animals were divided into three lots, each lot consisting of six animals. The cattle comprising each lot were inoculated respectively with suspensions prepared in physiological saline, 1 per cent saponin and 2 per cent cholesterol in liquid petrolatum.

Two animals in each lot received single injections of 40×10^9 organisms suspended in the respective excipients. The remaining four in each lot were inoculated, at four different sites, with a constant number of organisms (40×10^9), suspended in the same excipient. All injections were made subcutaneously, in clipped and sterilized areas anterior to the shoulder, on both the right and left sides.

The procedure employed in determining the presence of viable Brucella at inoculation sites was identical with that employed in connection with similar studies in group 1. While the observations in this group are incomplete at this time and data submitted necessarily of a preliminary nature, the available information tends to

[7]Haring, C. M., and Traum, J.: Personal communication.

verify and complement, in part, the results of a similar procedure carried out in the preceding animal-inoculation studies.

RESULTS

Observations relative to the local and general reactions noted in these animals have coincided to such an extent with one another, and with those of the original group, as to make a detailed discussion unnecessary.

The persistence of local reactions was comparable in all respects with that observed in animals of group 1, inoculated with similar suspensions. In most cases, the reactions at the undisturbed sites were definitely regressive, 30 days following inoculations.

Uniform thermal reactions of 2 to 3° F. were observed in all cases. The maximum temperatures were recorded within 24 to 36 hours, after which they subsided to normal within a period of 72 to 84 hours.

Agglutination Titres.—Frequent post-injection blood agglutination tests were conducted for the purpose of detecting possible variations in agglutinin response. The results of these tests are summarized in table V. It will be observed that no significant differences are apparent in the reactions obtained over a period of 85 days following inoculation. Variations in titres appear to be no greater in animals of different lots than is observed in individuals injected with the same material.

In view of the trend of these reactions, it would appear doubtful whether any marked differences in the persistence of detectable agglutinins will be observed.

Recovery of Brucella from Vaccinal Sites.—The results of bacteriological examinations of material secured from inoculation sites are summarized in table VI. Up to the present time, these determinations have covered a period of 92 days following inoculation. It will be observed that viable Brucella cultures have been recovered from animals in each lot at 85 days. However, positive cultures were isolated at 92 days from only those animals which had received 2 per cent cholesterol in liquid petrolatum suspensions. It will be noted, in this connection, that the number of viable organisms present in the aspirated material was relatively high.

The relationship between the number of organisms isolated and the excipient employed closely parallels the results obtained with similar agents utilized in the original group. The persistence of vaccinal organisms at inoculation sites continues to be most pronounced in connection with 2 per cent cholesterol in liquid petrolatum mixtures. Furthermore, only slight variations in the persistence of viability are apparent between the animals receiving physiological saline suspensions and those in which 1 per cent saponin was employed.

RABBIT INOCULATIONS

Further studies relative to the effect of various excipients on localization of vac-

TABLE VI—Recovery of viable Br. abortus from vaccinal sites. Group 2—Yearling steers.

Days Subsequent to Injection										
15	22	29	36	43	50	57	64	71	78	85	92
Approximate Number Viable Br. Abortus per cc.											
Group 1—1% Saponin											
$30x10^3$	$10x10^3$	$1.4x10^3$	$5.3x10^3$	175	75	45	0	0	45	45	0
Group 2—2% Cholesterol—Liquid Petrolatum											
$25x10^3$	$43x10^3$	$57x10^3$	$6x10^3$	$8.5x10^3$	$6x10^3$	$7x10^3$	$10x10^3$	$2x10^3$	$4x10^3$	$4.2x10^3$	$5.8x10^3$
Group 3—Saline											
$8.5x10^3$	$7x10^3$	$2.1x10^3$	$6x10^3$	$1x10^3$	0	90	30	30	30	30	0

0—No viable Brucella observed.

cinal injections were conducted on a group of inoculated rabbits. For this purpose a total of 48 ten-month-old rabbits were used. Fifteen animals in each of three lots were injected on the same date with *Br. abortus* strain 19 suspended in physiological saline, 1 per cent saponin and 2 per cent cholesterol in liquid petrolatum, respectively. Uniform 2-cc. injections of 16 x 10^9 organisms, suspended in various excipients, were injected subcutaneously in depilitated areas posterior to the shoulder.

For the determination of agglutinin response, three additional rabbits each received 1-cc. injections of 8 x 10^9 organisms suspended in the respective excipients.

At frequent intervals following inoculation, one animal from each lot was destroyed and a detailed study made of the vaccinal sites in regard to size, weight, and tendency for localization. Weight and size determinations were made on the extirpated processes. Bacteriological examinations for the presence of *Br. abortus* were conducted on material secured from inoculation sites as well as other tissues and organs throughout the body. Cultural procedure employed in these determinations was similar to that previously described.

Size and Character of Local Reactions.—A comparison of local reactions, at varying intervals following inoculation, is outlined in table VII. These determinations are based on existing differences in size and weight of extirpated vaccinal processes.

A high degree of mortality was encountered in the saponin-injected rabbits. Approximately 50 per cent of these animals died within a period of 48 hours following inoculation. Further indication of the marked toxicity of this material is apparent in the fact that spontaneous rupture and sloughing occurred at inoculation sites in two of the surviving rabbits.

A comparison of the results obtained in autopsied rabbits over a period of 55 days, further emphasizes the marked persistence of local reactions encountered in the use of 2 per cent cholesterol in liquid petrolatum suspensions. It will be observed that in three instances, complete disappearance of the vaccinal injections was noted in saline injected rabbits, destroyed at intervals of 34, 44 and 50 days following inoculation. While the persistence of local reactions in the saponin-injected animals is somewhat greater than in the case of saline injections, a distinct variation still exists, when compared with cholesterol in liquid petrolatum inoculations.

Prompt localization of vaccinal injections was most prominently displayed in those rabbits in which 2 per cent cholesterol in liquid petrolatum were employed as an excipient. Very little evidence of gravitation of the inoculum was observed in animals inoculated with this material. Furthermore, encapsulation was well marked in these rabbits 19 days following injection. In contrast to these observations was the tendency for extensive gravitation of vaccinal material containing 1 per cent saponin. Evidence of this was apparent in the fact that connective tissue proliferation, in the form of tracts, was consistently present in saponin-injected animals. In most cases, these tracts extended 6 to 8 cm. ventral from injection sites.

The material secured from early developing processes has uniformly presented a white, semi-fluid appearance. As regression of the local reactions take place, gradual liquefaction and absorption occur.

Agglutination Reactions.—A comparison of the blood agglutination reactions observed in these animals is shown in table VIII. The results of these determinations fail to reveal any significant variations associated with the use of different excipients. It appears possible that a maximum titre may be more rapidly acquired in connection with the use of saline suspensions than in the case of 1 per cent saponin or 2 per cent cholesterol in liquid petrolatum mixtures. Likewise, there is some indication that detectable agglutinins may persist over a longer period in animals inoculated with 1 per cent saponin and 2 per cent cholesterol in liquid petrolatum mixtures.

Bacteriological Determinations.—The results of bacteriological examinations of inoculation sites, conducted on autopsied rabbits, is summarized in table IX. While

these determinations cover a period of only 55 days following inoculation, striking differences are apparent in the persistence of viable *Br. abortus* in these areas. Negative results obtained at 50 and 55 days in rabbits receiving saline and saponin suspensions are in direct contrast to the recovery of viable Brucella at the same time from rabbits in which 2 per cent cholesterol in liquid petrolatum was employed as an excipient.

Routine bacteriological examinations conducted on other tissues at the time of autopsy have been for the most part negative. *Br. abortus* was isolated from the internal iliac and precrural lymph glands of rabbit No. 136, destroyed 19 days following inoculation. Positive cultures were likewise secured from the precrural lymph glands of rabbit No. 119, destroyed 34 days after inoculation. Both of these rabbits received original injections of saline suspended vaccine. Positive Brucella cultures were also obtained from the internal iliac and precrural lymph glands of rabbit No. 122. This animal had been injected with a 2 per cent cholesterol in liquid petrolatum suspension 19 days previous to autopsy. The results of similar examinations conducted on tissues other than vaccinal sites have been negative up to the present time.

VIRULENCE DETERMINATIONS ON BRUCELLA CULTURES ISOLATED FROM INOCULATION SITES IN CATTLE

The availability of Brucella cultures isolated at varying intervals from vaccinal sites prompted the investigation of possible

TABLE VII—Comparison of local reactions observed in rabbits injected with uniform amounts of Br. abortus strain 19 suspended in various excipients.

RABBIT NO.	EXCIPIENT	DIMENSIONS AND WEIGHTS OF EXTIRPATED LESIONS REMOVED AT INDICATED INTERVALS FOLLOWING INOCULATION					
		DAYS					
		19	25	34	44	50	55
		DIMENSIONS IN CM.—WEIGHTS IN MG.					
136	Saline	2.8x1.9x9 6,100					
147	Saline		3.5x2x.9 2,175				
119	Saline			0			
280	Saline				0		
140	Saline					0	
109	Saline						2x1.8x.8 2,280
122	2% cholesterol liquid petrolatum	6.2x2.6x.7 12,125					
143	2% cholesterol liquid petrolatum		5x2.5x1.5 6,450				
126	2% cholesterol liquid petrolatum			3.5x2.8x2 7,975			
148	2% cholesterol liquid petrolatum				3x2x1.5 4,975		
102	2% cholesterol liquid petrolatum					6.5x3x1.5 16,920	
127	2% cholesterol liquid petrolatum						4x2.2x1.8 7,290
150	1% saponin				6.5x1.8x6 4,750		
181	1% saponin					5x2.5x1 8,720	
116	1% saponin						#0

Note: Uniform vaccinal injections of $16x10^9$ organisms employed throughout.
0—Complete absorption.
#—Lesion had ruptured ten days previously.

TABLE VIII—Blood agglutinin response in inoculated rabbits.

Rabbit No.	Excipient	Amount Injected	Agglutination Titre at Indicated Intervals Following Injection							
			Days							
			0	13	19	25	34	44	50	55
			Maximum Dilution Showing Complete Agglutination							
108	2% cholesterol liquid petrolatum	1 — cc. $8x10^9$	Neg. 1:25	800	1,600	3,200	3,200	1,600	1,600	3,200
134	1% saponin	1 — cc. $8x10^9$	Neg. 1:25	3,200	3,200	3,200	3,200	800	1,600	3,200
113	Saline	1 — cc. $8x10^9$	Neg. 1:25	800	1,600	800	800	400	400	400
			Autopsied Animals							
136	Saline	2 — cc. $16x10^9$	Neg. 1:25		1,600					
147	Saline	2 — cc. $16x10^9$	Neg. 1:25			800				
119	Saline	2 — cc. $16x10^9$	Neg. 1:25				800			
280	Saline	2 — cc. $16x10^9$	Neg. 1:25					400		
140	Saline	2 — cc. $16x10^9$	Neg. 1:25						800	
109	Saline	2 — cc. $16x10^9$	Neg. 1:25							1,600
122	2% cholesterol liquid petrolatum	2 — cc. $16x10^9$	Neg. 1:25		1,600					
143	2% cholesterol liquid petrolatum	2 — cc. $16x10^9$	Neg. 1:25			1,600				
126	2% cholesterol liquid petrolatum	2 — cc. $16x10^9$	Neg. 1:25				1,600			
148	2% cholesterol liquid petrolatum	2 — cc. $16x10^9$	Neg. 1:25					400		
102	2% cholesterol liquid petrolatum	2 — cc. $16x10^9$	Neg. 1:25						1,600	
127	2% cholesterol liquid petrolatum	2 — cc. $16x10^9$	Neg. 1:25							3,200
150	1% saponin	2 — cc. $16x10^9$	Neg. 1:25					1,600		
181	1% saponin	2 — cc. $16x10^9$	Neg. 1:25						400	
116	1% saponin	2 — cc. $16x10^9$	Neg. 1:25							400

changes in virulence that might occur in *Br. abortus* strain 19 under these conditions. Virulence determinations were based on the results of guinea pig inoculations.

Ten cultures recovered from vaccinal sites at intervals of 59 to 97 days following inoculation were utilized for comparison with stock cultures of *Br. abortus* strains A-69 and 19. The preparation of saline suspensions from 48-hour growths was carefully controlled to insure the administration of a uniform number of organisms in all cases.

Seventy-two male guinea pigs of uniform size and age were selected for use in these determinations. Six animals were inoculated with each culture studied. All guinea pigs received uniform subcutaneous injections of 2 x 10^9 organisms suspended in 0.25 cc. physiological saline.

The results of these inoculations were determined on the autopsied guinea pigs six weeks subsequent to inoculation. The spleen, liver and testes were examined in each case for the presence of macroscopic lesions. These observations, together with

weights of all spleens and the results of spleen cultures, were recorded.

RESULTS

A composite summary of results obtained in these determinations is indicated in table X. It will be noted that no evidence of enhanced virulence has been demonstrated in any case. Slight variations observed in agglutination titres and spleen weights appear to have little significance in connection with the cultures studied. The differences noted in the percentage of infected spleens is commonly encountered in groups of guinea pigs inoculated with *Br. abortus* strain 19. Susceptibility of guinea pigs is indicated by the extensive lesions observed in the control group inoculated with *Br. abortus* strain A-69.

Comparative studies relative to the immunological response induced in calves vaccinated with *Br. abortus* strain 19, suspended in physiological saline and 2 per cent cholesterol in liquid petrolatum, are incomplete at this time and will be reported at a later date.

DISCUSSION

It should be emphasized that the purpose of these experiments was essentially an attempt to ascertain whether a retarded rate of vaccinal absorption might be secured through the use of selected excipients. Furthermore, it was believed that this procedure might in itself offer promise for further investigation into the possibility of higher antigenic values being induced in this manner.

That certain pertinent observations have resulted from this study, which has dealt primarily with the duration of viability of *Br. abortus* at vaccinal sites, is apparent in the fact that the results have in all cases been conditioned to a considerable extent by the excipients employed. Thus, it was found that the persistence of viability could be increased from 85 days, as observed in connection with saline suspended vaccines, to at least 150 days by the use of 2 per cent cholesterol in liquid petrolatum as an excipient.

Considerable difference has been noted in the bactericidal action of various excipi-

TABLE IX—Recovery of viable Br. abortus from vaccinal sites. Inoculated rabbits.

Rabbit No.	Excipient	Bacteriological Examinations at Indicated Intervals — Days Following Inoculation — Results in Viable Brucella per cc.					
		19	25	34	44	50	55
136	Saline	$63x10^3$					
147	Saline		$25x10^3$				
119	Saline			10^3			
280	Saline				650		
140	Saline					0	
109	Saline						0
122	2% cholesterol liquid petrolatum	$82x10^3$					
143	2% cholesterol liquid petrolatum		$57x10^3$				
126	2% cholesterol liquid petrolatum			$21x10^3$			
148	2% cholesterol liquid petrolatum				$10x10^3$		
102	2% cholesterol liquid petrolatum					$12x10^3$	
127	2% cholesterol liquid petrolatum						$2x10^3$
130	1% saponin				860		
131	1% saponin					0	
116	1% saponin						0

0 indicates no Br. abortus isolated.

TABLE X—Virulence determinations on cultures isolated from vaccinal sites at various intervals following inoculation with Br. abortus strain 19.

Culture No.	Interval in Days Between Inoculation and Recovery of Culture	Source of Culture		Observations on Autopsied Guinea Pigs		
		Cow No.	Excipient Employed	Average Blood Titre	Average Spleen Weights, Mg.	Percentage Infected Spleens
1	59	2,014	1% saponin	1:25	773	16.6
2	65	1,978	Saline	1:50	671	60.0
3	65	2,011	100% oil of sweet almond	1:100	863	16.6
4	65	1,997	6% cholesterol oil of sweet almond	1:100	885	33.3
5	65	1,988	50% lanolin liquid petrolatum	1:100	855	0
6	65	2,012	4% cholesterol oil of sweet almond	1:50	836	0
7	72	1,987	6% cholesterol liquid petrolatum	1:100	733	16.6
8	72	1,980	4% cholesterol liquid petrolatum	1:100	796	0
9	65	2,008	2% saponin	1:50	881	0
10	97	1,999	2% cholesterol liquid petrolatum	1:100	734	60.0
Controls						
11	Br. abortus (bovine) strain 19			1:100	993	33.3
12	Br. abortus (bovine) strain A-69			1:800	2,338	100.0

0 indicates no Br. abortus isolated.

Note—All guinea pigs received uniform injections of $2x10^9$ organisms.

ents on *Br. abortus*. This has been most prominently displayed as the result of *in vitro* determinations, where extreme variations have been observed. This diversity has ranged from a highly toxic effect, seen in the complete sterilization within 96 hours of Brucella cultures suspended in 50 per cent lanolin-liquid petrolatum mixtures, to the opposite extreme, where such excipients as 100 per cent liquid petrolatum may preserve viability over a period of several months.

The recovery of viable Brucella from inoculation sites for a maximum of 85 days following subcutaneous inoculation of saline suspended vaccine seems quite remarkable in view of the relatively short interval during which it is possible to demonstrate viability in organisms of other genera similarly injected.

Confirmatory evidence regarding the stability of reduced virulence exhibited by *Br. abortus* strain 19 has been demonstrated in a series of ten cultures recovered from injection sites in different animals over a period of 97 days. These determinations, based on the results obtained in carefully controlled guinea pig inoculations, have without exception failed to reveal any evidence of enhanced virulence.

Summary

1) Experiments designed primarily for the purpose of evaluating the action of various agents employed as excipients in *Brucella abortus* vaccines are described. These studies have included the *in vitro* as well as the *in vivo* behavior of viable Brucella organisms in such excipients as liquid petrolatum, oil of sweet almond, lanolin, saponin, cholesterol and physiological saline.

2) Anticipated correlation between the action of various excipients on *Br. abortus in vitro* and their effect in animal tissues has not been possible. While 50 per cent lanolin in liquid petrolatum suspensions were sterilized within 96 hours at 37.5° C., it was possible to isolate viable Brucella from vaccinal sites 85 days following inoculation of identical suspensions.

3) No variations in general reactions, following animal inoculation, can be associated with the use of various excipients studied. Furthermore, the transient char-

acter of these reactions is similar to that observed in saline suspended injections.

4) Marked differences were noted in the persistence of local reactions following inoculation of *Br. abortus* suspended in various excipients. These reactions may persist for a period of at least five months in the case of 50 per cent lanolin-liquid petrolatum mixtures, in contrast to the rapid absorption which commonly occurs within three to four weeks following the administration of comparable bacterial suspensions prepared in physiological saline.

5) *Br. abortus* strain 19 may remain viable at the site of inoculation for at least 85 days following subcutaneous injection of saline suspended vaccine. This persistence of viability may be increased to at least 150 days by the use of 2 per cent cholesterol in liquid petrolatum as an excipient.

6) No significant differences were noted in the agglutinin response induced in cattle and rabbits as the result of *Br. abortus* inoculations, in which various excipients were employed.

7) In no instance was any evidence of enhanced virulence observed in ten cultures recovered at intervals of 59 to 97 days from vaccinal sites in cattle inoculated with *Br. abortus* strain 19.

8) Based on the theory that increased immunological response may be induced by retarded antigen absorption, 2 per cent cholesterol in liquid petrolatum has shown sufficient promise to justify further study.

The Efficiency of Soaps and Other Disinfectants in Destroying Mastitis Streptococci*

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THE MARKED and consistent histological changes observed by Petersen, Hastings and Hadley[1] in udders which had secreted milk free from streptococci or other microörganisms commonly associated with mastitis, but which was abnormal when tested by chemical means, point strongly to the possibility of a nonspecific factor or a virus being associated with streptococci in the production of bovine mastitis. This hypothesis is supported by the recent report by Broadhurst, Cameron and MacLean[2] of the isolation of a filtrable virus from the blood and milk of cows with mastitis.

Even though the published reports of these and other investigators have cast serious doubt upon the rôle of mastitis streptococci as the primary etiological agents in the majority of cases of bovine mastitis, [1, 2, 3] the present consensus is that *Streptococcus agalactiae* or related mastitis streptococci play an important rôle in clinical mastitis.

Although a virus may be the primary etiological factor in most cases of bovine mastitis, it probably serves mainly as a precursor of streptococcic infection. Petersen and Hastings[3] have found that, "Nonspecific mastitis apparently lowers the resistance of the mammary gland to infection with *S. agalactiae*. *S. agalactiae* can be quite easily transmitted to quarters affected with nonspecific mastitis by external infection of the teat."

A number of workers have confirmed the ease with which streptococci can be transmitted to susceptible mammary glands although it is not known whether nonspecific factors were present in the udders used in their experiments. Little[4] found that, "the udder of the cow could be infected by multiple inoculations of small numbers of actively growing nonhemolytic streptococci introduced *via* the teat canal." He found that the ability of streptococci to produce mastitis depended upon the number passing beyond the meatus of the teat.

Several experiments are reported in which only a small percentage of infections followed when pure cultures or even badly infected milk was placed upon the external orifice of the teat. It should be remembered, however, that under natural herd conditions, the opportunity for contamination of the teat by the milker occurs twice each day over long periods and there are repeated opportunities for fly contamination. Injury to the external sphincter or a temporary negative pressure during certain parts of the act of milking may contribute to the entrance of streptococci.

It is logical, therefore, to assume that contamination of the teat sometimes would occur when the resistance of the udder was at a minimum, a condition not likely to be duplicated in a large proportion of the experimental inoculations where only a limited number of trials are made.

The tendency of bovine mastitis to spread through a herd, often attacking cows in the order in which they are milked, points to the importance of milking in the spread of this disease. A practical and convenient

*Scientific paper No. 452, College of Agriculture and Agricultural Experiment Station, State College of Washington.

[1]Petersen, E. H., Hastings, E. G., and Hadley, F. B.: The pathology of nonspecific mastitis and consideration of possible etiological agents. Cornell Vet. xxviii (1938), pp. 307-324.

[2]Broadhurst, Jean, Cameron, Gladys, and MacLean, M. Estelle: A filtrable virus isolated from mastitis cows. Cornell Vet. xxix (1939), pp. 261-270.

[3]Petersen, E. H., and Hastings, E. G.: A study of the possible relationship between nonspecific mastitis and streptococci infection of the bovine udder. Cornell Vet. xxix (1939), pp. 11-24.

[4]Little, Ralph B.: Bovine mastitis I. The significance of the dose factor in the production of experimental mastitis. Cornell Vet. xxvii (1937), pp. 297-308.

method of destroying the mastitis streptococci on the hands of the milkers and in the droplet of milk remaining at the end of the teat after milking, which is attractive to flies, is significant in the control of bovine mastitis.

To be applicable, the disinfectant must possess unusual characteristics. In addition to being effective and rapid in its action against streptococci in the presence of small amounts of milk, it must be nonirritant to the hands of the milkers and the teats of the cows even when used routinely over long periods of time. In addition, it must be nonpoisonous and not esthetically offensive so that there would be no objection to its use around milk; and it must be without odors which might be absorbed by milk. It also should be stable so that deteriorated supplies would not be used, readily available, economical, and preferably not covered by patents that increase the cost.

ACTION OF CHLORAMINES SLOW

In the past, various chlorine compounds, especially the hypochlorites, have served fairly well. However, tests have shown the chloramines, especially those which are mixed with alkalies to produce a commercial product, to be too slow in exerting their germicidal action.

The same criticism also applies to the alkaline hypochlorites and especially those which are strongly buffered at a high pH by combination with substances such as trisodium phosphate. Hypochlorites of low alkalinity are effective and rapid germicides and have given excellent results in many cases even though they are not stable in the presence of milk. A few dairymen have discontinued their use, claiming that their long-continued application leads to skin irritation. It is probable that individual cows differ in their susceptibility to skin irritation from the hypochlorites.

Search was made for some type of compound which would be effective against streptococci and suitable for frequently-repeated applications to the hands of the milkers and the teats of the cows. The soaps and related compounds were found to be effective germicides against mastitis streptococci and to meet the other requirements. (See charts 1 and 2.)

EXPERIMENTAL

The Food and Drug Administration method for testing disinfectants[5] was modified to furnish data applicable to this particular problem. The tests were conducted at 40° C. rather than at 20° C. because it was assumed that this would be approximately the temperature of the warm wash water used. Five per cent of skim milk was added to provide additional organic matter such as would be the case when the wash water became contaminated with milk from the hands or teats. While subcultures were taken at the end of one, two, as well as at the end of five and ten minutes, as in the official method, the results of exposure to the disinfectant solution for one minute are the most applicable to this problem, since under conditions of actual application, one minute would be the maximum exposure which the milker could be induced to make.

The cultures used in these tests were grown for 24 hours at 37.5° C. in F.D.A.[5] broth, pH 6.8. Each loop was found by dilution to contain more than 10,000,000 and less than 100,000,000 viable inocula except in the case of culture No. 6 where a loop of dilutions of 10,000,000 usually failed to initiate growth but dilutions of 1,000,000 uniformly were positive. After admixture with the disinfectant solution, each loop represented more than 1,000,000 viable inocula except in the case of culture No. 6 in which the number was between 100,000 and 1,000,000. The subcultures were made into fresh skim milk to which had been added sufficient brom-cresol-purple to tint, 0.1 per cent peptone and sufficient NaOH to give a final reaction of pH 6.8 after the milk had been tubed and autoclaved. Growth was readily detected by the formation of sufficient acid to change the color of the brom-cresol-purple.

The subcultures were read at the end

[5]Ruehle, G. L. A., and Brewer, C. M.: United States Food and Drug Administration methods of testing antiseptics and disinfectants. U. S. Dept. of Agriculture Cir. No. 198 (1931).

of one, two and seven days of incubation at 37.5° C. Subcultures of organisms exposed to soaps frequently were apparently negative at the end of one day and positive at the end of two days. Only rarely did subcultures of soap-treated organisms show

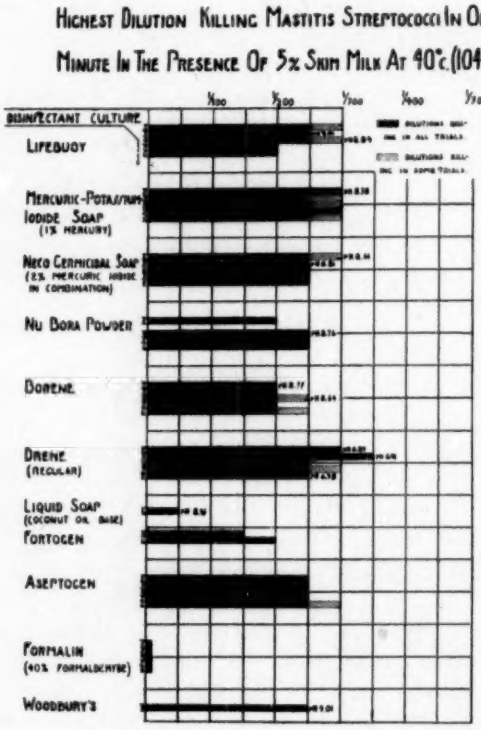

Chart 1.

negative at the end of two days and positive at the end of seven days. This would indicate the absence of bacteriostatic action of soaps, as with the organisms used in these tests very small numbers of viable inocula would require more than one day of incubation to produce sufficient acidity in the milk to be recognized.

In the tests with formalin, however, bacteriostatic action was evident and subcultures frequently were apparently negative at the end of two days and positive at the end of five days.

THE CULTURES

The five cultures of mastitis streptococci used were all of bovine origin and belonged to type "B" of Lancefield's[6] classification when typed according to the technic described by Brown.[7] Cultural reactions showed cultures 2 and 6 to be alpha prime *S. agalactiae*, and cultures 3, 4 and 5 to be *S. asalignus* which differs from *S. agalactiae* in not utilizing salicin. On blood agar, cultures 2, 5 and 6 were found to produce alpha prime hemolysis, 4 the beta type, and 3 was found to be a gamma. After several months, the hemolytic power of culture 4 diminished.

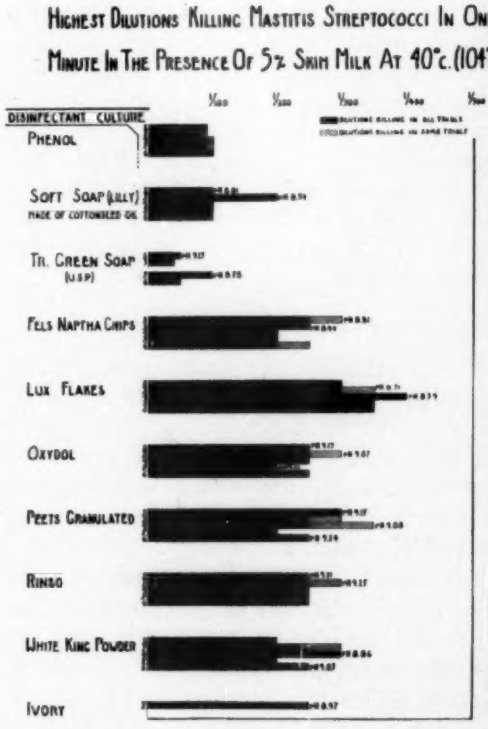

Chart 2.

In the absence of the 5 per cent skim milk and at 40° C. the soaps were germicidal against mastitis streptococci in considerable higher dilutions. With exposures of five minutes, dilutions of 1:1,250 to 1:1,750 of most of the soaps usually killed the strains tested. Trisodium phosphate 1:100, which was pH 10.86 when mixed

[6]Lancefield, Rebecca C.: A serological differentiation of human and other groups of hemolytic streptococci. J. Exper. Med. lvii (1933), pp. 571-595.

[7]Brown, J. Howard: A simplified method of grouping hemolytic streptococci by the precipitin reaction. J. A. M. A. cxi (1938), pp. 310-311.

TABLE I—Phenol—40° C.

Dilution of Phenol	No Organic Matter Added — Subcultures After				Dilution of Phenol	5 Per Cent Skim Milk Added — Subcultures After			
	1 Min.	2 Min.	5 Min.	10 Min.		1 Min.	2 Min.	5 Min.	10 Min.
1:100	− ǂ − − ǂ	− − − − −	− − − − −	− − − − −	1:90	− ǂ − − −	− − − − −	− − − − −	− − − − −
1:110	+ + + + +	− ǂ − + +	− − − − −	− − − − −	1:100	+ + ǂ ǂ −	+ + − − −	− − − − −	− − − − −
1:120	+ + + + +	+ + + + +	ǂ + − + +	− − − − −	1:110	+ + + + +	ǂ + + + ǂ	− − ǂ − −	− − − − −
1:130	+ + + + +	+ + + + +	ǂ + + + +	− − − − ǂ	1:120	+ + + + +	+ + + + +	− ǂ ǂ − ǂ	− − − − −
1:140	+ + + + +	+ + + + +	+ + + + +	− − − − +	1:130	+ + + + +	+ + + + +	ǂ + + ǂ +	− − − − −
1:150	+ + + + +	+ + + + +	+ + + + +	+ + + + +	1:140	+ + + + +	+ + + + +	+ + + + +	ǂ + + + ǂ

Cultures 2, 3, 4, 5 and 6. Cultures 2, 3, 4, 5 and 6.

— Indicates no growth in subculture, hence less than one viable inoculum of survivors was present in each loop.

+ Indicates growth in subculture, hence the survival of at least one viable inoculum in each loop.

ǂ Indicates growth in subculture in some trials and absence of growth in other trials.

TABLE II—Low alkalinity hypochlorite.

B – K Powder 50 Per Cent Available Chlorine	P. P. M. Av. Cl.	5 Per Cent Skim Milk Added — Subcultures After			
		1 Min.	2 Min.	5 Min.	10 Min.
1:5,000	100	− + − + −	− − − + −	− − − − −	− − − − −
1:6,250	80	+ + + + +	+ + + + +	− − − + −	− − − + −
1:8,333	60	+ + + + +	+ + + + +	+ + + + +	+ + + + +
1:12,500	40	+ + + + +	+ + + + +	+ + + + +	+ + + + +

Cultures 2, 3, 4, 5 and 6.

TABLE III—Azamine.

Azamine* Compound Azamine 12-14% Sodium Chloride 2.91% Lactose 84.95%	5 Per Cent Skim Milk Added — Subculture After			
	1 Min.	2 Min.	5 Min.	10 Min.
1:166	8 of 14+	5 of 14+	2 of 14+	2 of 14+
1:200	7 of 14+	3 of 14+	4 of 14+	1 of 14+
1:250	6 of 15+	4 of 15+	7 of 15+	2 of 15+
1:333	10 of 15+	7 of 15+	5 of 15+	5 of 15+

Cultures 2, 3, 4, 5 and 6.

*Tolyl-azo-diamino-pyridine hydrochloride.

TABLE IV—Sodium hydroxide.

Household Lye 97.5% Sodium Hydroxide	pH	5 Per Cent Skim Milk Added			
		Subcultures After			
		1 Min.	2 Min.	5 Min.	10 Min.
1:300	11.22	+++++	---+-	-----	-----
1:350	11.21	+++++	#-#++	-----	-----
1:400	11.20	+++++	#++++	-----	-----
1:500	11.18	+++++	+++++	-####	-----

Cultures 2, 3, 4, 5 and 6.

with milk and broth, failed to kill any of the cultures in ten minutes. This was much more alkaline than any of the soap solutions tested.

Summary and Conclusions

Solutions of commercial soaps and soap powders, at 40° C. and in the presence of 5 per cent skim milk and 5 per cent broth culture of the organisms, were found to be between two and three times as effective in killing mastitis streptococci in one minute as was phenol and were equally as effective as 100 parts per million of the most actively germicidal of several hypochlorites tested.

A soap containing cresols was no more germicidal than were the nonmedicated soaps, and the soaps containing mercury compounds were only slightly more effective.

A soft soap usually called "green soap," tincture of green soap, and a coconut oil base liquid soap were found to be inferior germicides against mastitis streptococci.

No correlation was observed between the germicidal efficiency of the mixture of soap solution, milk and broth and the degree of alkalinity. Drene, said to consist largely of triethanolammoniumlauryl sulfate, which gave a final dilution near the neutral point, was effective in dilutions comparable with the solid soaps.

High-test household lye, which probably kills by the hydroxyl-ion concentration produced, was found to be effective in dilutions of 1:300 and 1:500, which gave a final alkalinity of pH 11.22 to 11.18, but was much slower than the soaps in exerting germicidal action.

Soap solutions in the concentrations usually obtained in lathering the hands with soap in warm water are effective disinfectants against mastitis streptococci, and may satisfactorily replace other disinfectants for the hands of the milkers and the teats of the cows.

Household lye is the disinfectant of choice for dairy barns where it is especially necessary to destroy mastitis streptococci on the floors.

Bovine Type of Tuberculosis in Sheep*

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OWING to the fact that tuberculosis in sheep is of rare occurrence only limited numbers of cases have been made available for studies by investigators; and of all the cases studied, in which the causative organisms were typed, a very large percentage have been found to be due to the avian type of the tubercle bacillus. All of the cases studied by Harshfield and Roderick[1] and all but one studied by Harshfield, Roderick and Hawn[2] were found to be the avian type of tuberculosis; the one exceptional case referred to was not definitely identified. The bovine type of tuberculosis in sheep, therefore, appears to be exceedingly rare. Apparently, few cases definitely typed as bovine have been encountered. One such case was reported by Van Es and Martin[3] and two cases were found by Jowett.[4] More recently a case was reported by Stubbs and Live.[5] For a number of years studies have been made of tuberculosis in sheep, in the laboratory of the pathological division of this bureau[6, 7, 8, 9, 10] with particular reference to the type of infection, and it is interesting to note that out of a total of 60 cases typed 59 were found to be of the avian type, and the one exception typed bovine. It is this one exceptional and rather interesting case that is made the subject of this report.

The tissues from this particular case of suspected tuberculosis in a sheep were received from Inspector-in-charge J. A. Thompson, West Fargo, N. D. The tissues consisted of liver, lungs, and a number of lymph glands. The autopsy report, accompanying the tissues submitted, stated that slight lesions were observed in the cervical, bronchial, mediastinal prescapular, precrural, popliteal, supramammary, internal iliac and renal lymph glands. The lesions were slight in the liver and of the miliary type in the lung. The lesions were chronic in character and partially calcified. Difficulty was experienced in demonstrating acid-fast organisms in smear preparations from the different tissues, giving evidence that the organisms were quite scarce in the calcified lesions. Histologically, the lesions were found to be typical of tuberculosis.

ANIMAL INOCULATIONS

Two rabbits, 2 guinea pigs and 2 chickens were inoculated with an emulsion made from composite material from the lesions in several of the lymph glands. These, and subsequent animal inoculations, were made intraperitoneally.

After a period of approximately two months the chickens were tested, with avian tuberculin, with negative results. The chickens, rabbits, and guinea pigs were then destroyed and autopsied.

Autopsy Findings.—In one of the rabbits lesions typical of tuberculosis were found in the lungs and associated lymph glands, on the pleura and in the kidneys. No lesions of tuberculosis could be demonstrated in the other rabbit, in the guinea pigs or chickens.

In view of these rather unusual inoculation results, in which lesions were found in one rabbit while none were found in the guinea pigs, and in order to obtain more definite information with regard to the type of infection in this case, two more guinea pigs and two more chickens were

*Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture.

[1] Harshfield, G. S., and Roderick, L. M.: J. A.V.M.A., lxxxv (1934), pp. 597-610.

[2] Harshfield, G. S., Roderick, L. M., and Hawn, M. C.: J.A.V.M.A., xci (1937), pp. 323-329.

[3] Van Es, L., and Martin, H. M.: Nebraska Agr. Exp. Sta. Bull. 49 (1930), p. 68.

[4] Jowett, W.: J. Comp. Path. and Ther., xli (1928), p. 255.

[5] Stubbs, E. L., and Live, I.: J.A.V.M.A., xcv (1939), pp. 173-176.

[6] Annual Report, Chief of Bureau of Animal Industry, 1936.

[7] Ibid., 1937.

[8] Ibid., 1938.

[9] Ibid., 1939.

[10] Unpublished data.

inoculated with an emulsion made from the kidney lesions of the rabbit, in which numerous acid-fast organisms had been demonstrated.

The two guinea pigs died at the end of nine and ten weeks, respectively, and generalized lesions of tuberculosis were found in both animals. Numerous acid-fast organisms were demonstrated in the splenic lesions of both guinea pigs. Culture media, 3 per cent glycerine agar and Dorset's egg medium, were seeded with material from the splenic lesions of one of the guinea pigs.

After two months the chickens were tested, with avian tuberculin. Both chickens gave positive results to the test. The chickens were retested after four weeks, with both avian and mammalian tuberculin. One of the chickens gave a well-marked reaction to the mammalian tuberculin, while the test results in the other were rather questionable. However, when subjected to a third test, three weeks later, with both types of tuberculin, both chickens gave well-marked reactions to the mammalian tuberculin, and were found negative to the avian tuberculin. Following the last test the chickens were destroyed and autopsied. No lesions of tuberculosis were found in either chicken.

After a period of about two months several of the tubes of Dorset's egg media, inoculated from the spleen of guinea pig, previously referred to, yielded pure cultures of an acid-fast organism, characteristic of the tubercle bacillus, from which subcultures were made.

CULTURE INOCULATIONS

As a further test it was decided to inoculate rabbits, guinea pigs, and chickens with the culture obtained from the guinea pig spleen. A suspension was subsequently made of the organisms from one of the subcultures, consisting of a small loop of the organisms in 6 cc. of normal saline solution. Each of two rabbits and two chickens received approximately 1 cc. each, and each of three guinea pigs received 0.5 cc. of the suspension of organisms.

All of the guinea pigs succumbed in less than three weeks, with well-marked and generalized lesions of tuberculosis. After 2½ months both rabbits exhibited considerable emaciation, indicating probable tuberculous infection, and were destroyed and autopsied. In one of the rabbits typical lesions of tuberculosis were found in the lungs, numbers of the lymph glands, omentum, peritoneum, and testes. In the other rabbit there were also well-marked lesions of tuberculosis in the lungs and associated lymph glands, small pinhead lesions in the spleen and kidneys, and small nodular lesions scattered over the omentum and large intestine. Smear preparations made from different lesions, in the guinea pigs and rabbits, revealed numerous acid-fast organisms.

At the end of a 2½-month period the two chickens inoculated with the culture appeared to be in a healthy condition, and were then tested with avian tuberculin. Both chickens gave slight reactions to the test. The chickens were held four weeks longer and again tested, with both avian and mammalian tuberculin. Both chickens gave very definite reactions to the mammalian tuberculin, with no definite evidence of reactions to the avian tuberculin. The chickens were then destroyed and autopsied. The autopsies failed to reveal any tissue changes suggestive of tuberculosis in either of the chickens. The results thus obtained through rabbit and guinea pig inoculations, and the apparent immunity of the inoculated chickens to both tissue and culture inoculation, would seem sufficient evidence that the tubercle bacillus involved in this case of sheep tuberculosis was of the bovine type.

The rather unusual, or peculiar, inoculation results obtained in the first group of animals, in which one of the rabbits proved susceptible to the tuberculous infection while the guinea pigs remained free from infection, is not readily explained. One possible explanation, however, would be that the virulence of the tubercle bacilli had been greatly reduced as a result of passage through the sheep, and also the

probability that the one rabbit affected was particularly susceptible. This would seem rather probable in view of subsequent inoculation results, indicating increased virulence of the organism after one passage through the rabbit, in which all guinea pigs inoculated readily succumbed to the infection with generalized lesions of tuberculosis.

This case is reported chiefly because of the apparent extreme rarity of the bovine type of tuberculosis in sheep.

A Note Concerning the Influence of Zinc on the Growth of Tubercle Bacilli on Malate Media*

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CULTURE MEDIA containing ammonium malate as a source of nitrogen have been used in these laboratories for the cultivation of *Mycobacterium tuberculosis* since 1928.[1] These media have been employed chiefly in studies of the nutrition of tubercle bacilli and as one result of the studies a malate medium was described[2] which afforded yields and final pH values approximating those afforded by the asparagine medium used here to produce tuberculin. With the increasing scarcity of asparagine brought about by war conditions, attention was redirected toward the malate medium as a possible substitute for the asparagine medium. However, the rapid and abundant growths formerly afforded by the malate medium were not obtained; instead growth was slow and the yields, with occasional exceptions, were much below expectations.

There seemed to be a possibility that the malate medium as formerly prepared had contained, as an unrecognized impurity, some unknown constituent which was essential for growth and that this ingredient was absent in the medium as now prepared. In a search for such a substance it was found, as shown in table I, that the addition to the medium of zinc, either alone or in combination, caused an increase in the rate and amount of growth and that the amounts of zinc required were quite small.

All of the media which contained added zinc sulfate, either alone or in combination with other salts, afforded considerably greater yields than the corresponding media without that salt. Since sulfur is a regular constituent of the medium, being present in the form of magnesium sulfate, the growth-promoting element would appear to be zinc. Doubling the amount of zinc present, which was only 0.4 mg., had no further effect; the effect of smaller amounts has not been studied.

Incidentally, the asparagine medium used to produce tuberculin was found to contain zinc, 24 mg. of that metal having been found in 100 Gm. of asparagine. Whether the malate medium prepared with distilled water contains zinc has not been definitely ascertained. A very slight trace may be present and this trace may account for the yield of about 1 Gm. of bacilli usually obtained from 100 cc. of that medium. Whether zinc is an essential element for the growth of *M. tuberculosis* can be determined only by using absolutely zinc-free media and experiments along this line have been begun.

*Bureau of Animal Industry, U. S. Department of Agriculture.

[1] Henley, R. R., and LeDuc, P. W.: Ammonium malate as source of nitrogen for tubercle bacilli in cultures. Amer. Rev. Tuberc., xxii (1930), p. 568.

[2] Ibid.: Growth and metabolism of tubercle bacilli. xl (1939), p. 313.

TABLE I.—Yields of tubercle bacilli from malate media containing different salts.

MEDIUM SALT ADDED	GRAMS DRY BACILLI PER 100 CC. CULTURE FLUID AT THE 42ND DAY		
	PN STRAIN*	C STRAIN*	DT STRAIN*
Control	1.10	1.08	0.63
Salt mixture No. 1†	1.87	1.76	1.60
Salt mixture No. 2†	1.89	....	1.83
$CaCl_2$ 10 mg. per 100 cc.	1.06	.86	....
NaCl 10 mg. per 100 cc.	.82	.78	....
$ZnSO_4$ 1 mg. per 100 cc.	1.86	1.66	1.89
$MnCl_2$ 1 mg. per 100 cc.	1.18	.87	1.09

*Weights given are an average of two cultures of the PN and DT strains and of one culture of the C strain.

†To each 100 cc. of the medium there was added 1 cc. of solution containing 1 Gm. $CaCl_2$, 1 Gm. NaCl, 0.1 Gm. $MnCl_2$ and 0.1 Gm. $ZnSO_4$ per 100 cc. No. 1 solution was 4 weeks old; No. 2 freshly prepared.

In so far as known, zinc has never been previously recognized as having any influence on the growth of *M. tuberculosis* on artificial culture media. Zinc, however, has long been known to have a stimulating effect on the growth of *Aspergillus niger*, its influence, according to Steinberg,[3] having been first recognized by Raulins.

[3]Steinberg, R. A.: A study of factors influencing the stimulating effect of zinc sulfate on the growth of *Aspergillus niger*. Memoirs of the Torry Botanical Club, xvii (1918), p. 287.

The Source and Availability of Infective Oöcysts in an Outbreak of Coccidiosis in Lambs in Nebraska Feedlots*

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DURING a study of an outbreak of coccidiosis in feeder lambs in central Nebraska during November and December, 1939, data were obtained which throw considerable light on certain problems in the epizoölogy of this disease under feedlot conditions. Additional data were obtained to show that lambs are infected upon arrival at the feedlots from the West, confirming similar observations by Thorp[1] and Deem and Thorp[2]. Information was obtained which indicates that old feedlots are probably not important in the development of feedlot coccidiosis, a question raised but not solved by Deem and Thorp[2]. Since it was apparent that the infection was brought in by the lambs themselves, a search was made for viable sporulated or infective coccidial oöcysts from water supply, feed materials and sites about occupied feedlots, in order to determine how the infection was acquired by lambs in sufficient magnitude to cause widespread clinical coccidiosis. An effort was made to evaluate water supply, feed materials and types of feedlot manure as media for the accumulation, sporulation and preservation of viability of oöcysts, and to determine the probable relationship of these factors to the development of this outbreak of coccidiosis. This study seemed advisable because there has been no previous convincing attempt to correlate the development of clinical coccidiosis in feeder lambs with the availability of infective oöcysts in the environment and feed materials.

Although more than 80,000 lambs were being fed at this establishment at the time the writer conducted his investigations, explorations to determine the source and availability of infective oöcysts were largely confined, for convenience, to one yard having 20 feedlots, averaging 800 lambs per lot. These 20 lots, containing approximately 16,000 lambs from New Mexico, were filled during one week and since there was little or no shifting of animals during the first few weeks of confinement, the lambs in the yard passed through a period of clinical coccidiosis at about the same time. Death losses from coccidiosis, together with widespread scouring and heavy oöcyst discharge in the feces, began after 12 to 16 days of feeding. Heavy death loss ceased after the clinical symptoms had lasted for about two weeks, but scours continued for a few to several weeks longer in many lambs. During the outbreak in these 16,000 lambs, there were 540 deaths from coccidiosis alone, this representing a mortality of 3.4 per cent. In addition, 1,563 scouring, emaciated lambs were removed to a hospital lot for special diet and care.

MATERIALS AND METHODS

This outbreak appeared to be intimately connected with feeds and feeding which, therefore, were given considerable attention. Seventeen of the 20 lots of lambs involved were fed corn silage and whole alfalfa throughout the entire epizoötic of coccidiosis, while only three lots of cull lambs were fed a fattening mixture of chopped alfalfa, molasses and ground corn from the beginning of the feeding period. The usual procedure was to maintain lambs on silage and whole alfalfa during the first several weeks of feeding, changing to the fattening mixture after a third to half of the feeding period had passed. Since coccidiosis struck after 12 to 16 days of confinement and feeding, the lambs were predominantly on silage and whole alfalfa during this critical

*Zoölogical Division, Bureau of Animal Industry, U. S. Department of Agriculture.

[1] Thorp, Frank Jr.: Some feedlot diseases of lambs. Vet. Med., xxxiii (1938), pp. 442-444.

[2] Deem, A. W., and Thorp, Frank Jr.: Variation in numbers of coccidia in lambs during the feeding season. Vet. Med., xxxiv (1939, pp. 46-47.

time. However, the three lots of cull lambs that were started entirely on the chopped alfalfa mixture also developed coccidiosis at the same time and the potential danger of this feed material as an enrichment medium for infective oöcysts could not be overlooked. No attempt was made at sanitation either inside feedlots or in feeding methods. No bedding was made available to lambs. Each feedlot was equipped with a shelter for protection of lambs from wind. Feeds were given in open troughs and additional amounts added to unused portions. No attempt was made to clean the feeding devices.

To determine whether lambs were infected upon arrival at the feedlots, fecal material was collected as pooled or individual samples from eight shipments of lambs immediately upon or shortly after their arrival from the West. The pooled samples were obtained to show the approximate average level of oöcyst discharge in entire shipments of lambs, and individual samples to show the incidence of infection. Representative fecal specimens from 6 of the shipments were obtained by pooling two pellets from random fresh droppings from each of 5 to 40 lambs from each shipment. Two of these pooled samples were collected from shipments of lambs, one from New Mexico and one from Colorado, which had been pastured on cut-over hay fields in the vicinity of the feedlots for about six weeks before their removal to the lots. The other pooled samples were taken from shipments of New Mexico lambs which were moved directly from the freight cars to the feedlots, three of the samples being obtained at the unloading yards and one at the feedlots on the day following confinement. All individual fecal samples consisted of single pellets from the rectum of each of twelve animals from two shipments from New Mexico, and were collected immediately upon their arrival at the unloading yards. Dilution oöcyst counts or sugar flotations, or both, were made on all fecal specimens to determine the incidence and magnitude of the coccidial infection at the time the lambs entered the feedlots. This enumeration technic was a volumetric dilution count of oöcysts per cc. of feces, and was carried out entirely in 15 cc. graduated centrifuge tubes on 0.5 to 1.0 cc. amounts of whole fecal sediment diluted with 14 volumes of tap water. The methods were crude, but were well adapted to the field conditions under which this study was made.

The feedlot materials examined for oöcysts were collected from all possible locations, as follows: a) sediment from the bottom of a typical watering trough; b) caked manure from several locations on the surface of a manure heap in a feedlot unoccupied since the preceding spring; c) a mixture of crushed dirt and manure, scraped from the bottoms and corners of feed troughs which also had not been used since the preceding feeding season; d) pulverized, dried feces and whole, dried fecal pellets from the ground of occupied feedlots; e) fecal pellets from among whole alfalfa, corn silage and a mixture composed of chopped alfalfa, molasses and ground corn in several types of feeding devices in occupied feedlots. All specimens were thoroughly crushed in a mortar, mixed into a paste with water and subjected to flotation in 15 cc. centrifuge tubes by mixing approximately 1 cc. quantities of the paste with 14 cc. amounts of a 40 per cent sugar solution. After sufficient time to permit flotation of oöcysts, the surface films of these tubes were removed with clean coverslips and studied. Oöcysts found were recorded as sporulated, unsporulated or too altered by drying for the contents to be recognized, and they were described to indicate their approximate state of desiccation and probable viability.

OBSERVATIONS

Oöcysts in Feces of Lambs upon Arrival at Feedlots.—Examinations of pooled fecal samples from six shipments of lambs immediately after their arrival at the freight yards or one day after confinement to the feedlots revealed oöcysts by dilution count or flotation in every sample. Only one of the pooled specimens representing averages for the entire shipments was negative by dilution count, but this sample showed

TABLE I—Oöcyst discharge in the feces of feeder lambs immediately upon or shortly after arrival at feedlots, showing the magnitude and incidence of the infection.

Shipment	Date (1939)	Origin of Lambs	History After Arrival	Time of Examination	Description of Fecal Specimens Examined	Average Number Oocysts per cc. of Feces	Results of Flotation
1	10–26	Colo.	Pastured in vicinity for 6 weeks before entering feedlot	2nd day in feedlot	22 pooled specimens, 2 pellets per lamb	900	None made
2	10–28	N. Mex.		Immediately upon arrival	40 pooled specimens, 2 pellets per lamb	2,400	do
3	11–2	do	Moved directly from freight cars to feedlot	2nd day in feedlot	20 pooled specimens, 2 pellets per lamb	0	+
4	11–3	do		Immediately upon arrival	7 pooled specimens, 2 pellets per lamb	1,200	None made
5	11–5	do		do	5 pooled specimens, 2 pellets per lamb	1,200	do
6	11–7	do		do	Single pellet do do do do do do	No count made do do do do do do	+ + + + + + +
7	11–16	do		do	do do do do do	300 300 300 0 0	None made do do + +
8	12–5	do	Pastured in vicinity for 6 weeks before entering feedlot	2nd day in feedlot	10 pooled specimens, 2 pellets per lamb	600	None made

oöcysts by flotation (table I, shipment 3). Average discharges in the other five pooled samples varied from 600 to 2,400 oöcysts per cc. of feces. These counts were fairly typical for healthy adult sheep (table I, shipments 1, 2, 4, 5, and 8). Pasturing on cut-over hay fields in the vicinity of the feedlots for several weeks apparently had no effect on the average level of the oöcyst discharge. Two lots of lambs had average discharges of 900 and 600 oöcysts per cc. of feces, respectively, at the time they were confined to the feedlots (table I, shipments 1 and 8). All individual fecal pellets taken from twelve lambs from the other two shipments showed oöcysts by dilution count or flotation (table I, shipments 6 and 7).

Oöcysts in Feedlots.—Fecal material from various points on the surface of a manure heap in a feedlot not occupied since the previous April or May, and therefore having been subjected to the desiccating effects of the summer, revealed a few extremely wrinkled, distorted and discolored oöcysts, all of which were unsporulated and showed signs of protoplasmic disintegration and death (table II, examination 1). No oöcysts were found in one examination of crushed manure and dirt which was scraped from the bottoms and corners of feed troughs not used since the previous spring (table II, examination 3).

No oöcysts were found in one examination of debris collected from the bottom of a drinking trough in a feedlot occupied by lambs at the height of the clinical period of coccidiosis.

In two batches of dried fecal pellets collected from several locations on the ground of occupied feedlots there appeared to be a heavy mortality in the contained oöcysts as a result of drying. In the first examination, about half of the oöcysts found were unsporulated and about an eighth of them could be recognized as sporulated, while the remainder were so badly discolored and distorted from the effects of desiccation that it was impossible to distinguish whether or not they were sporulated (table II, examination 4). The second examination showed similar results, roughly a fourth of the oöcysts being unsporulated, slightly more than a third sporulated, and the remainder dried to the point where

TABLE II—Occurrence and condition of oöcysts in fecal material from the ground and among feeds in feedlots.

Examination	Date (1939)	Description of Material		Unsporulated Oocysts*		Sporulated Oocysts*		Oocysts in Which Contents Were Unrecognizable as a Result of Excessive Wrinkling and Discoloration Through Drying*
		Nature	Age and Source	With Normal Appearance	Wrinkled, Distorted or Discolored Through Drying	With Normal Appearance	Wrinkled, Distorted or Discolored Through Drying	
1	10–29	Crusted manure from surface of dung heap	6-8 months; from lot not used since previous spring	0	10	0	0	0
2	11–3	Dry, hard fecal pellets	2-3 weeks; from beneath whole alfalfa in trough	0	17	0	0	0
3	11–3	Crusted manure-dirt mixture	6-8 months; from feed boxes not used since previous spring	0	0	0	0	0
4	11–8	Miscellaneous dried fecal pellets	2 days-4 weeks; from ground of lot occupied for 4 weeks	0	22	0	6	19
5	11–8	Pulverized, dried manure	Few days-4 weeks; from ground of lot occupied for 4 weeks	9	3	0	3	4
6	11–9	Moist, soft fecal pellets	1 day-6 weeks; from among corn silage in feed trough	6	3	9	0	0
7	11–9	Miscellaneous dried fecal pellets	Few days-6 weeks; from ground of lot occupied for 6 weeks	0	7	0	9	9
8	11–9	Hard, dried fecal pellets	1-7 days; from among chopped alfalfa-molasses-ground corn mixture in feed trough	1	5	1	2	1
9	11–14	Hard, dried fecal pellets	Few days- 3 weeks; from among hay leaves beneath whole alfalfa in trough	1	0	2	0	0
10	11–17	Moist, soft fecal pellets	1 day-2 weeks; from among corn silage in feed trough	4	1	28	8	0
11	11–17	Hard, dried fecal pellets	1 day-2 weeks; from among chopped alfalfa-molasses-ground corn mixture in feed trough	0	5	6	22	2

*Numbers represent total oöcysts counted in one cover-slip preparation from sugar flotation on 1 cc. of fecal sediment.

recognition of contents was impossible (table II, examination 7). All oöcysts showed the discoloring, distorting effects of drying, many having twisted and broken shells, but sporulation had occurred in a significant portion of them despite rapid drying.

In one examination of dried pulverized manure gathered from the ground in several places about a feedlot occupied for four weeks, about half of the oöcysts found were unsporulated and appeared fairly normal, while the remainder were about equally divided between wrinkled, discolored, unsporulated and sporulated oöcysts and those too dried for determination (table II, examination 5).

Dried fecal pellets obtained from among the hay leaves and dirt that had accumulated beneath whole alfalfa in a feed trough not used for the preceding two weeks revealed only wrinkled, distorted unsporulated oöcysts (table II, examination 2). Dried pellets from a similar location in

a feed trough which had been in an occupied feedlot for three weeks prior to the time of examination revealed one normal unsporulated oöcyst and two normal sporulated oöcysts (table II, examination 9).

In the first examination of fecal pellets found among corn silage in feed troughs in occupied feedlots, half of the contained oöcysts appeared normal and fully sporulated, while most of the remainder were normal and unsporulated, with only a few showing slight signs of drying (table II, examination 6). In the second examination, two-thirds of the oöcysts from pellets found among silage were normal and sporulated, a fifth of them were sporulated but showed some signs of desiccation, while the remainder were unsporulated (table II, examination 10).

Two groups of fecal pellets were collected in occupied feedlots from feed troughs containing a mixture consisting of chopped alfalfa, molasses and ground corn. In the first examination, only a tenth of the oöcysts observed appeared sporulated and normal, a fifth were sporulated but badly distorted and discolored through drying, while the remainder of the oöcysts were either not sporulated or were too dried for the contents to be recognized and nearly all showed the effects of rapid drying (table II, examination 8). The second examination showed that four-fifths of the oöcysts were sporulated, but only about a fifth of the total appeared normal, the others showing the effects of desiccation (table II, examination 11).

DISCUSSION AND OBSERVATIONS ON THE SOURCE AND AVAILABILITY OF INFECTIVE OÖCYSTS

The observations suggested that old feedlots were probably effectively sterilized by the heat and drought of the previous summer and were therefore noninfective to lambs at the beginning of the fall feeding season. The failure to find sporulated oöcysts, or unsporulated oöcysts that appeared viable, in over-summered fecal material from a manure heap, and the failure to find any oöcysts in crusted manure and dirt from feed troughs subjected to summer heat, indicate the sterilizing effect of summer temperatures and desiccation. Although meager in scope, these observations seemed to warrant the conclusion that over-summered feedlots were sterilized before the arrival of the fall feeder lambs and played, therefore, no part in the present outbreak of coccidiosis. This opinion is supported by the fact that severe coccidiosis previously developed at this feeding establishment in new feedlots on land never before used for feeding, indicating that the lambs occupying the lots were themselves responsible for the infection. Since old feedlots were removed as possible sources of the feedlot infection, the obvious conclusion was that feeder lambs carry the infection at the time they are placed in the feedlots.

This conclusion was verified by the finding of oöcysts in feces from every group of lambs and individual newly arrived lamb examined. It appeared from these observations that probably every lamb of every shipment was discharging small numbers of coccidial oöcysts at the time of arrival at the feedlots and that fresh fecal pellets from these lambs constituted the source of the oöcysts which were responsible for the infection that resulted in widespread clinical coccidiosis 12 to 16 days after the beginning of confinement and feeding. The problem, therefore, consisted in determining how these oöcysts deposited by the lambs inside feedlots became available in sufficient numbers in infective form to produce the epizoötic of coccidiosis.

The answer to this problem necessitated a search for sporulated oöcysts in drinking water and among all feed materials, since the development of clinical coccidiosis depends upon the ingestion of viable sporulated oöcysts, as Johnson[3] has demonstrated experimentally in studies on chicken coccidiosis. This search for sporulated oöcysts involved observations of ways in which water and feeds were contaminated with feces. In order to determine the probable

[3]Johnson, W. T.: Coccidiosis of the chicken with special reference to species. Oregon Agr. Exp. Sta. Bull. 358 (1938), pp. 3-33.

rôle of water and various feeds in the development of the present outbreak it was necessary to evaluate each as a medium for the sporulation and preservation of viability of oöcysts.

Drinking troughs throughout the entire feeding establishment were kept nearly filled or overflowing with fresh well water, and were located so that overflow occurred outside of the feeding area. Lambs therefore had an ample supply of fresh, clean water which in nearly all cases was obtained while animals stood upon dry ground. Occasionally the water was slightly contaminated with fecal pellets carried upon the wool and with manure dust distributed by the wind, but these were removed as possible sources of infection either by being washed away in the overflow or by sedimentation, since coccidial oöcysts soon settle out in water when washed free of fecal matter. Although no oöcysts were located in one examination of bottom debris, it is probable that small numbers of oöcysts gradually accumulate at the bottoms of the troughs. Such oöcysts, even if they succeed in sporulating in that environment, may be eliminated as possible contributors to the feedlot infection as long as the water level is maintained well above the bottoms of the troughs, since they are several inches removed from the drinking surface. It was the practice in these feedlots to keep troughs filled or overflowing with water, and it is not likely that the water supply played any part in the development of this outbreak of coccidiosis.

Fecal pellets picked up at random from the ground, after having undergone desiccation for at least a few days to several weeks at the time they were collected, contained significant numbers of sporulated oöcysts despite the heavy mortality that resulted from extreme drying in this exposed location. The possible infectivity of the sporulated oöcysts contained in these dried pellets is not definitely known, but since hard, dry pellets were not observed to be extensively carried to the feed materials on the wool of lambs or by wind, it was not considered likely that such pellets played a part in the feed contamination that led to this outbreak of coccidiosis.

Most of the fecal pellets deposited in the feedlots were ultimately pulverized by the feet of the lambs and dried into a light dust which was easily distributed over the premises by wind. Examination showed that this manure dust contained considerable numbers of sporulated oöcysts, but since virtually all of these oöcysts would have been subjected to extreme drying before they could be distributed to the feed in any significant quantity of the wind, it is probable that most of them would be nonviable by the time they could be available to the animals in the feed. Moreover, wind action was fitful and irregular, and there was no relation between possible windborne contamination of feed and the regular precision with which clinical coccidiosis developed in the feedlots in question.

Whole alfalfa was fed at this feeding establishment by maintaining large amounts adjacent to the outside of the feedlot panels bordering one or two sides of the lots, and occasionally in racks inside the feedlots. Both methods of feeding hay required that lambs reach between horizontal boards to obtain the feed, and were thus designed to prevent trampling of feed with feet or an appreciable amount of fecal contamination in other ways. Nevertheless, it was not difficult to locate fecal pellets among the dirt and hay leaves that had accumulated beneath the coarser stems of the alfalfa. These fecal pellets which gained access to whole alfalfa were subjected to fairly rapid desiccation, and invariably soon became dry and hard in the arid autumn climate that was prevalent in Nebraska at that time. Despite rapid drying, some of the oöcysts contained in these pellets succeeded in sporulating and hence were probably potentially infective for lambs. However, the likelihood that the contamination of whole alfalfa constituted a significant source of infection was considered remote. Since alfalfa is relatively coarse, stemmy and dry, nearly all pellets and manure dust which find their way into it sift quickly through the feed and form part of the ac-

cumulation beneath. This fecal material, even though containing numbers of apparently infective oöcysts, probably does not constitute an infective hazard to lambs, as long as sufficient alfalfa is kept available to prevent the animals from eating significant amounts of the dried debris underneath the hay. Since it was the practice to feed liberal quantities of whole alfalfa at the feedlots involved, making it unnecessary for lambs to eat far beneath the surface, this method of feeding could not be incriminated in the present outbreak of coccidiosis.

It was the practice at these feedlots in feeding corn silage to replenish the feed in troughs twice daily, adding additional amounts to the uneaten portions remaining from previous feedings. No attempt was made to clean periodically the feeding devices, so that there was a gradual accumulation of uneaten silage beneath the fresh portions that were continually added. After feedlots had been occupied for a few days, it was not difficult to find whole or fragmented fecal pellets among the silage, and since this feed material was always moist and compact, the fecal material was scattered fairly uniformly throughout. In nearly all cases the pellets found here were moist and soft and easily crushed between the fingers, indicating that they were fresh at the time they gained access to the feed, and that the moisture of the silage preserved the natural moisture of these pellets. Some of the manure found among the silage consisted of fragments of pellets, demonstrating that pellets may disintegrate and the manure become widely scattered throughout such feed. Laboratory tests conducted by Christensen,[4] have shown that fecal pellets retaining their natural moisture provide nearly optimum conditions for sporulation of oöcysts, an observation confirmed in the present study by the discovery of considerable numbers of normal, sporulated oöcysts in the moist pellets found among the silage. There appears to be no doubt that the silage fed in the manner described permitted the gradual accumulation of fecal material, favored the sporulation and retention of viability of the contained oöcysts by virtue of its moisture content, and thus provided a rich inoculum of infective oöcysts for susceptible lambs fresh from the range. Since at least two or three days are required under optimum conditions for complete sporulation of oöcysts of most species of sheep coccidia, this infective inoculum must therefore be available to lambs after three or four days of feeding and gradually increase in volume as feeding continues.

Most of the silage was fed in long troughs extending the entire length of the lots along the outside of the boundary panels, making it necessary for lambs to reach through the panels to obtain the feed. Some silage was fed in 2′x16′ troughs which were placed inside the feedlots, and also designed to require lambs to reach between horizontal boards for the feed. Since the slits for head space in both devices were designed to accommodate only the heads of average lambs, it was at first difficult to see how an appreciable amount of feed contamination could occur. Closer observation, however, showed that small or extremely active lambs frequently found their way into troughs and dropped pellets there. Lambs frequently transported fresh pellets, which readily adhered to the wool, from the bedding areas to feed materials. Occasionally, manure was carried to the feed upon the hoofs of crowding lambs attempting to find space at the feeders. These sources of contamination, together with the absence of any attempts to clean feeding devices periodically, resulted in the gradual accumulation of fecal material throughout the silage.

The mixture of chopped alfalfa, molasses and ground corn was fed to three lots of cull lambs in troughs identical to those used in feeding the corn silage, and feeding was

[4] Christensen, John F.: Sporulation and viability of oöcysts of *Eimeria arloingi* from the domestic sheep. J. Agr. Res., lix (1939), pp. 527-534.

done in the same manner. This mixture was highly palatable, firm, compact, and slightly sticky but not moist. Considerable fecal contamination occurred in the same ways as were noted for silage, and the bulkiness of the material prevented contaminating fecal pellets from sifting through the feed. After this mixture had been available for several days in well-filled troughs, it was not difficult to locate numbers of fecal pellets by searching through the feed. Pellets found in this feed were invariably hard and dry during the period covered by this study, indicating that the mixture did not contain moisture sufficient to prevent fairly rapid drying of the fecal pellets that were carried to the feed by the lambs. Despite desiccation, sporulation of oöcysts in considerable numbers occurred before the pellets containing them were completely dried. It is not known just how long these sporulated oöcysts remain viable inside these dried pellets, but considering the fact that the lambs which were fed entirely on the chopped alfalfa, molasses and ground corn mixture showed an increase in average oöcyst discharge during the first two weeks of feeding and developed clinical coccidiosis with some mortality, it appeared that this type of feed might constitute a significant factor in the development of coccidiosis if fed during the first few weeks of the feeding period in the manner described. Considerable numbers of oöcysts inside these dried pellets remained unsporulated, however, and probably many of the sporulated oöcysts were soon rendered nonviable as a result of drying, so that the possibilities that this feed would build up as massive an inoculum of infective oöcysts as was noted for the naturally moist silage seemed improbable.

The outbreak of coccidiosis that prompted this study occurred during a fall characterized by extremely dry weather. Days were repeatedly fair, with occasional high winds. Throughout November and the first half of December, corn silage was the only feed material used that contained enough moisture to prevent fairly rapid drying of fecal pellets, and it was in these moist pellets that the greatest numbers of normal, sporulated oöcysts were found. Sporulated oöcysts were located in most of the more or less dried fecal pellets found among other feeds, but the mortality from desiccation among such oöcysts was presumably great. The chopped alfalfa, molasses and ground corn mixture was fed from the beginning of the feeding period to only three lots of lambs, but since these also developed clinical coccidiosis it was apparent that this feed material made considerable numbers of viable sporulated oöcysts available to susceptible lambs. Since 17 of the 20 lots of lambs involved in this outbreak were fed silage and whole alfalfa during the epizoötic, it appeared that the silage provided the principal culture medium for the enrichment of the inoculum of infective oöcysts that caused the present trouble.

Potentially every feed which is given in the open becomes a favorable sporulating medium for oöcysts during periods of wet weather. Any chopped feed, by virtue of its compactness, will retain moisture for some time and therefore constitute a perfect environment for the development and preservation of oöcysts inside contaminating fecal pellets. In wet weather, whole alfalfa will still permit fecal pellets to sift through the network of stems to form part of the debris beneath, although conditions in this wet debris will likely be favorable for oöcyst development. These sporulated oöcysts in the accumulations beneath whole alfalfa will probably never be available to lambs in quantities approaching those in the chopped feeds, which keep fecal material almost uniformly suspended throughout. It is probable, also, that wet weather will accelerate the rate of contamination of feeds with manure, since wet pellets will be more easily transported to feeds on wool and feet of lambs. For these reasons, it appears that wet weather greatly enhances the dangers of serious outbreaks of coccidiosis if the moisture comes during the first few weeks of feeding, when lambs are

most susceptible to the attack of these parasites.

SUMMARY AND CONCLUSIONS

During the study of an outbreak of coccidiosis among 16,000 feeder lambs in central Nebraska, in which the mortality from the disease was 3.4 per cent, an effort was made to determine the source of infective oöcysts and to evaluate drinking water and various feed materials as media for the sporulation and preservation of oöcysts, and as contributors to the outbreak under consideration.

Observations indicated that the previous summer probably effectively sterilized the old feedlots and that fresh fecal pellets from lambs which had just arrived at the feedlots constituted the source of the oöcysts that led to the clinical infection. It was concluded that fecal contamination of the drinking water was negligible, that dried fecal pellets or dried pulverized manure from the ground of occupied feedlots did not materially contaminate feed materials, that fecal material gaining access to whole alfalfa was not readily accessible to the lambs, and that therefore these were not factors in the production of the epizoötic.

Corn silage, fed in open troughs that were never cleaned, provided ideal moisture conditions for the sporulation and preservation of viability of oöcysts in the fecal pellets that lambs carried to the feed. Since considerable numbers of normal, sporulated oöcysts were found in pellets that became well scattered among this feed, the silage was considered to be the main medium that was responsible for the oöcyst enrichment that led to the outbreak. A mixture consisting of chopped alfalfa, molasses and ground corn, fed during the critical first few weeks of feeding to only about 2,400 of the 16,000 lambs involved, permitted fairly rapid drying of contaminating pellets, but these pellets contained enough sporulated oöcysts to produce clinical coccidiosis in these lambs. That this mixture was a medium for the sporulation and accumulation of oöcysts was definitely indicated.

It was concluded that all chopped feeds fed in open troughs that are not cleaned periodically are increasingly dangerous as sources of coccidial infection in wet weather, since these bulky feeds retain moisture well and would, therefore, provide ideal conditions for the sporulation, accumulation and preservation of viability of oöcysts that get into feeds *via* fresh fecal pellets.

Studies on Thysanosoma Actinioides,* Part II†

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THE EXISTENCE of the cestode, *Thysanosoma actinioides*, has been known for more than 100 years. A number of parasitologists have endeavored to shed some light upon its life cycle, but little information on this subject is present in the literature.

According to the excellent review by Stiles and Hassal,[1] the parasite was first discovered in a Brazilian swamp deer by Natterer in 1823. Diesing described it in 1834 under the name of *Th. actinioides*. He changed the name to *Taenia fimbriata* in 1850. In 1891 Moniez encountered this cestode in Italy and named it *Moniezia fimbriata*. Stiles in 1892, after careful study, reëstablished the original designation of the parasite, *Th. actinioides*.

Faville[2] first reported the presence of tapeworms in Colorado sheep in 1885. Fringed tapeworms were not specifically mentioned, although a number of cestodes were reported as occurring in the bile ducts. During the following two years Curtice[3] reported in detail on a *T. fimbriata* epizoötic in Colorado. He mentioned the parasite again in 1890.[4] Hall in 1931[5] gave a nontechnical description of the tapeworm and its habitat but stated that the life cycle was unknown.

In 1931 Christensen[6] conducted autopsies on 1,200 feedlot lambs and concluded that the reputed great pathogenicity of fringed tapeworms was unfounded. Newsom and Cross[7] and Newsom and Thorp[8] after many years of investigation conclude that, even though flocks of lambs in feedlots may show a high percentage of infection by these tapeworms, the pathogenicity of the tapeworm seems to be overrated. Newsom[9] succeeded in obtaining and photographing[7, 8] free embryonated ova of *Th. actinioides*. Hall[10] reported the presence of eggs, evidently similar to those found by Newsom. The report lacks any description or illustration of these ova.

This cestode is described in all of the common textbooks of animal parasitology, but data on the reproductive physiology and the life cycle are lacking.

It is highly probable that an intermediate host is necessary for propagation of this cestode and that an invertebrate may play the rôle of a host to facilitate the cysticercoid stage. Krull[11] has shown this to be the case with the genus Moniezia. A small plant mite harbors the cysticerci of the broad tapeworm. The necessity of an intermediate host for the completion of the cycle of *Th. actinioides* and geographical limitations governing the habitat of this host are emphasized by the fact that the

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[1]Stiles, C. W., and Hassal, A.: A revision of the adult cestodes of cattle, sheep and allied animals. U. S. Bur. Anim. Indus. Bull. 4 (1893).

[2]Faville, N. H.: Report of Vet. Dept., Colorado State Agr. Col. (1885).

[3]Curtice, C.: Tapeworm disease of sheep of the western plains. Ann. report U. S. Bur. Anim. Indus. (1889), pp. 165-184.

[4]Curtice, C.: The animal parasites of sheep. U. S. Bur. Anim. Indus.

[5]Hall, Maurice C.: Parasites and parasitic diseases of sheep. U. S. Dept. of Agri. Farmers Bull. 1330 (1931-1932).

[6]Christensen, R. O.: An analysis of the reputed pathogenicity of *Thysanosoma actinioides* in adult sheep. J. Agr. Res. xlii (1931), pp. 245-249.

[7]Newsom, I. E., and Cross, Floyd: Feedlot diseases of lambs. Colorado Exp. Sta. Bull. 409 (1934).

[8]Newsom, I. E., and Thorp, Frank Jr.: Lamb diseases in Colorado feedlots. Colorado Exp. Sta. Bull. 448 (1938).

[9]Newsom, I. E.: Personal communication (1939).

[10]Hall, Maurice C.: The discharge of eggs from *Th. actinioides*. Proc. Helminth. Soc. Wash. I (1934), pp. 6-7.

[11]Krull, W. H.: On the life history of *Moniezia expansa* and Cittotaenia sp. (*Cestoda anaplocephalidae*). Proc. Helminth. Soc. Wash. vi (1939), pp. 10-11.

fringed tapeworm fails to reproduce itself in eastern pastures or feedlots among domestic sheep as well as among lambs imported from the Rocky Mountain region.

A review of the literature indicates that this tapeworm is only slightly, if at all, pathogenic for feedlot lambs, but since the worm occurs in the bile ducts of the liver this organ is condemned as unfit for food. Data collected by the Colorado Experiment Station show that out of 233 lambs autopsied during the 1939-40 feeding season 37.8 per cent of the livers contained fringed tapeworms. Out of 104 experimental lambs followed to the packing house, 67 (64.4 per cent) showed liver infection. Another experimental lot containing 363 lambs showed a liver infection of 31.6 per cent. Since liver condemnation constitutes a considerable economic loss it is of economic importance and biological interest to discover the phases of the life cycle of this parasite for the purpose of preventing infections.

EXPERIMENTAL

In the fall of 1938 three worm specimens were sent to the laboratory for identification. During the examination of one of these specimens which was identified as a segment of *Th. actinioides*, several encapsulated, oval bodies were observed under the same coverslip but outside the segment. These capsules contained from 5 to 12 embryonated ova which were in the hexacanth stage. The capsule was composed of fibrous connective tissue, one end being rounded, the other terminating in a frayed, tail-like structure (figs. 5, 6). Several egg cases were subjected to microdissection, and the ova could be readily liberated.

More than 100 worms were removed from a stock of preserved specimens, and the terminal segments were systematically examined for egg cases. None was found. From the terminal proglottids of a few of these worms, however, single embryonated ova were obtained which proved to be morphologically identical to those obtained from the previously mentioned egg cases and to those observed by Newsom[7, 8, 9] (fig. 7). Since the egg capsules were not observed in the segments, it was impossible to establish their relationship to this cestode.

A systematic search for these egg cases in the fringed tapeworm was continued throughout the remainder of the lamb-feeding season of 1938-39 and was initiated again at the beginning of the 1939-40 period. In numerous examinations of lamb feces in the fall of 1939, only one capsule, containing embryonated eggs, was found in one specimen. Fringed tapeworms were present in the small intestine of this lamb at autopsy. In the contents of the small intestines a few detached segments were found which contained embryonated eggs within egg cases.* From then on the entire intestinal contents were removed, the contents of the small gut being kept separate from those of the large gut. In cases where *Th. actinioides* was present in the small intestine and liver, repeated washing and decanting of the fecal material revealed from 1 to 30 detached segments which contained egg cases.

This procedure has been repeated 266 times, and 540 gravid segments have been recovered.

Bourne[12] and Gassner[13] have reported an improved and more sensitive method in fecal examination. This procedure made it possible to obtain egg cases from droppings of lambs (fig. 5). Out of 168 lambs submitted for autopsy, 113 harbored fringed tapeworms. The feces of 62 of the latter were positive for egg cases. The low recovery may be explained by the following facts:

1) Most of these lambs were dead from overeating or other causes. It appears that some of the worms detach themselves voluntarily or perish quickly during the illness and after death of the animal. The rather large numbers of tapeworms which were found in various stages of disintegration seem to support this view.

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*Hereafter the embryonated eggs within egg cases shall be referred to as "egg cases."

[12]Bourne, R. F.: The use of Babcock cream test bottles in the flotation of parasite eggs. J.A.V.M.A. xciii (1938), pp. 261-262.

[13]Gassner, F. X.: Studies in canine coccidiosis. J.A.V.M.A. xcvi (1940), pp. 225-231.

2) The large intestines and especially the rectum usually contain little material, because of diarrhea or the animals being off feed for some time.

3) The infection by tapeworms was low.

On the other hand lambs in good health examined at slaughter showed a much higher incidence. The livers and intestines of 16 tapeworm-infected lambs were recovered at the packing house and examined. One hundred and thirty-seven gravid segments were recovered. Of the 16 animals, 13 also harbored egg cases, as evidenced by fecal examinations. Composite fecal samples collected in the feedlots where these lambs were kept proved to be positive for egg cases in every instance. Whole segments were not found among the droppings; neither were free embryonated ova encountered at any time.

In light infections of the liver only, the worms were found to be small (up to 20 cm.) and their heads were located in the smaller ramifications of the bile duct system, deeply embedded in the liver substance. When the terminal portions of such worms did not extend through the common bile duct, only a few detached segments were found in the intestines. In heavy infections, however, when the common bile duct appeared distended by tapeworms and approximately one-fourth of the total length of some worms extended through the entrance of the bile duct into the duodenum in backward direction with the flow of chyme (fig. 1), a greater number of segments were recovered in every instance. The constricted terminal portion of such worms showed segments in various stages of gravidity, the last proglottid sometimes being in the act of detachment (fig. 2). Egg cases were not detected in the terminal segments of entire worms taken from the small intestines. However, a critical microscopic examination of the ovarian tract of such proglottids revealed embryonating ova in various stages of development (figs. 12, 13).

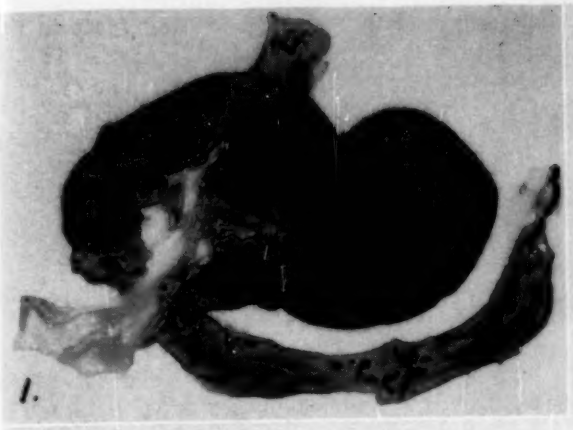
The contents of gall bladders were examined a number of times. While no free proglottids were observed, immature worms were found in two instances. The smallest one measured 5 mm. and showed a prominent scolex and five proglottids. It is difficult to say whether these young worms were in the process of ascending from the intestine to the liver or had lost their hold on the mucosa of the small bile duct and were carried to the gall bladder by the outflowing bile.

The lengths of *Th. actinioides* specimens found varied considerably. The majority of mature worms measured between 25 and 30 cm.; immature forms measured between 0.5 and 15 cm. Lamb No. 743, autopsied on January 20, 1940, harbored in its small intestine two specimens which measured 62 cm. each in length, their greatest width being 1.4 cm. The measurements were taken after the worms were permitted to relax normally in warm physiological salt solution, avoiding any stretching.

A description of the worm as a whole can be omitted here since the accounts of Stiles[1] and Curtice[3] were confirmed in general, with the exception of length as just mentioned. The anatomy of the terminal segments offers a number of interesting features. Each proglottid is hermaphroditic, containing two sets of sexual organs. Each set is composed of a male and female portion which are placed symmetrically in the segment, terminating near one of the bilateral genital pores. The testes develop

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE

Fig. 1. Lamb liver heavily infected with fringed tapeworms; note the worms in bile ducts and extending into duodenum. Fig. 2. Terminal segments, the last segment in the act of detachment (x 70). Fig. 3. Proglottid showing antero-median aperture (x 70). Fig. 4. Embryonating eggs in parauterine pouches in vivo (x 352). Fig. 5. Egg cases as seen in fecal sediment (x 70). Fig. 6. Egg case containing four embryonated eggs. Upper left: a free embryonated egg (x 352). Fig. 7. Free embryonated eggs; note clear, outer envelope (x 352).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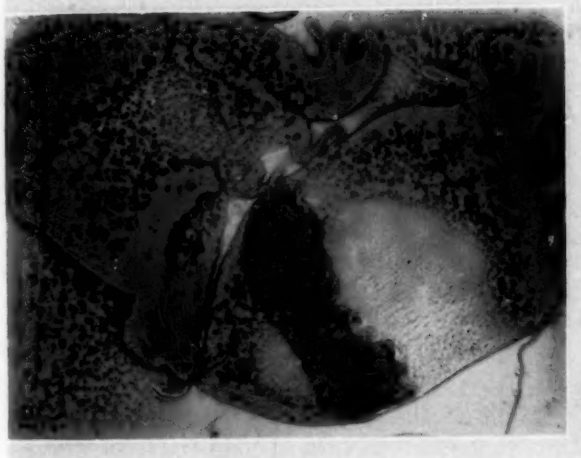
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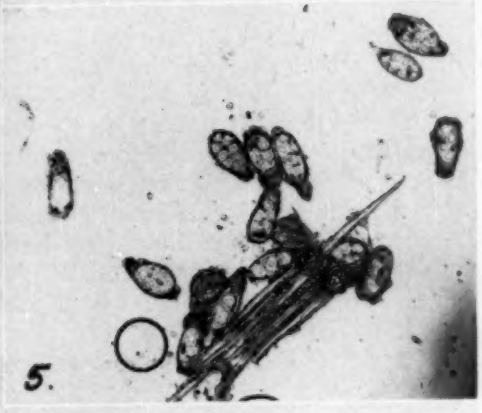
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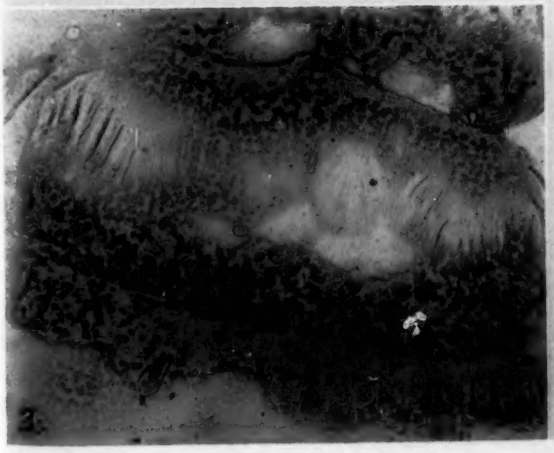
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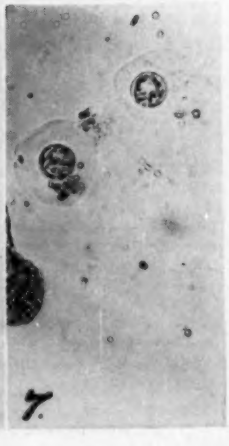
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early and are found to occupy the whole width of the posterior half of the segment. A large number of small sacs are connected by tubules, all of which terminate in the contorted efferent duct and ultimately in the seminal receptacle near the genital pore. The ovaries develop later and are situated on either side of the proglottid, just median to the longitudinal canals. While the cestode exhibits two sets of reproductive organs, it contains only one uterus. This is in disagreement with the findings of Curtice[3] who speaks of two uteri.

The single uterus consists of a narrow tube which traverses the entire width of the anterior portion of the segment in an undulating fashion, and connects with the ovaries on either side (figs. 8-11). It becomes increasingly visible in the segments of the posterior half of the worm. As the width of the last five or six segments decreases, their thickness increases, and the uterine convolutions become more distinct. Paraffin sections of the last five segments show that each of the anterior curvatures of the uterine convolutions connects with one or more parauterine pouches which are arranged in a fringe along the anterior aspect of the uterine tube (fig. 9). Each pouch shows a relatively heavy connective tissue wall, and its lumen, not patent in the nongravid proglottid, is continuous with the uterine tube proper through a narrow neck. The ova are presumably fertilized in the lateral termination of the uterine tube. During or after cleavage the developing ova pass through the slender necks into the parauterine pouches where the development of the embryos is continued (figs. 12, 13). Upon maturation the pouches contain five to twelve embryonated ova. The slender necks of these pouches are severed at the junction with the uterus proper, which has begun to degenerate. The egg cases are allowed to congregate in the anterior portion of the segment. It has been repeatedly observed that the embryonating ova are not discharged or liberated from the segment as such but, as observed earlier, are retained in the pouches which form the egg cases.

The detached segments attain various forms, depending upon the degree of their gravidity. Ordinarily, segments are not detached unless fully gravid. When observed in the fecal sediment, they may appear as b l u i s h - or yellowish-white, square, cylindrical, or semilunar bodies. The fully gravid segments become opaque because of the compactness of the egg cases. When such a gravid segment is placed upon a slide, covered with a coverslip, and examined under the microscope, egg cases may be observed in the reproductive tract. Upon application of gentle pressure to the coverslip over the segment, the egg capsules are observed emerging from the segment through an aperture located in its median anterior portion (fig. 3). They appear singly and by their long axis. Gravid segments that had been kept in the refrigerator in physiological salt solution for several days were removed to a warm room, placed under a coverslip on a glass slide, and subjected to strong light. Microscopic examination revealed that after about five minutes egg cases were emerging from the anterior aperture without the application of pressure of any kind except that possibly caused by the weight of the coverslip.

The egg cases appeared identical to those observed in 1938. The outside measurements of ten egg cases were 145.8 to 267.7 μ wide by 110.5 to 123.2 μ long. The cavity containing the embryos measured 75.5 to 96.2 μ by 50.8 to 60.6 μ. Structurally the embryonated ova show the embryo within a single shell which measures 11.55 μ in

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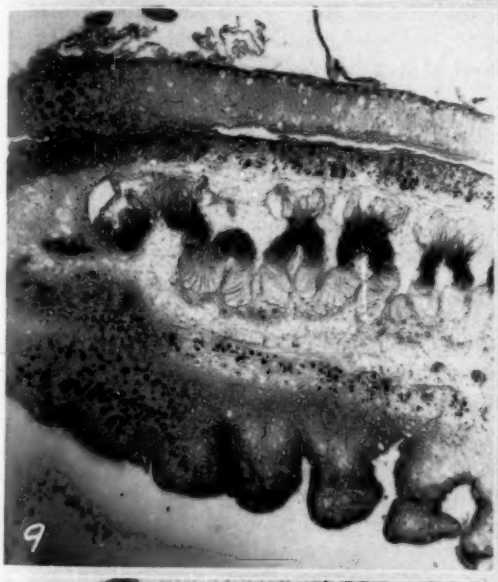
Fig. 8. Horizontal section of proglottids showing developing uterus (x 72). Fig. 9. Proglottid showing maturing uterus with parauterine pouches (x 72). Fig. 10. Same, further progression (x 72). Fig. 11. Same, showing embryonating ova (x 72). Fig. 12. Parauterine pouches, early gravid (x 360). Fig. 13. Parauterine pouches with developing embryos (x 360). Note—figures 1 to 7 are photographs made from fresh, unstained preparations. Figures 8 to 13 are photographs made from paraffin sections stained with a modified Mallory stain.



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diameter. These shells are remarkably uniform in size. The hexacanth is distinctly visible, and the individual hooklets measure, on the average, 3.85 μ in length. Surrounding the embryo proper is a colorless, corona-like envelope which is of nonrefractile, transparent material (fig. 7). The outside measurements were 19.25 to 26.95 μ. It is interesting to note that this extra-embryonic covering is very resistant to pressure and shows only a faint affinity for basic stains. While most parts of the segment reacted sharply to fixing and staining fluids, these clear envelopes seemed to be entirely unaffected. The pyriform structure common to embryos of the genus Moniezia is absent.

The heavy fibrous capsule enclosing the embryos, which is furnished by the uterine end organs of the worm, seems to afford an excellent protection for the young against external influences of various kinds. This was borne out by the results of a number of incubation trials which are to be reported elsewhere.

The bulk of gravid segments collected during the feeding season of 1939-40 were stored in physiological salt solution in the refrigerator beginning January 15, 1940. The salt solution has been renewed once a week and aeration of the cultures has been facilitated twice a week since then. Weekly examinations of the segments and their contents up to date have shown neither aggressive nor regressive changes of any kind, with the exception of the color of the cuticulum of the proglottids, which changed in some instances from white to a brownish-yellow. The egg cases show no changes in the fibrous capsules, and the embryos seem to be alive.

Feeding experiments, with newborn, sterile lambs, are under way at the present time to ascertain the possibilities of direct propagation of this parasite without an intermediate host.

SUMMARY

A historical review of the literature on *Thysanosoma actinioides* shows that little information on the reproductive physiology and the life cycle of the parasite has been gleaned during the more than 100 years its existence has been known. It is probable that an intermediate host, perhaps an invertebrate, is necessary for the propagation of this cestode.

While the pathogenicity of the fringed tapeworm to western feedlot lambs appears to be overrated, the heavy infection of livers and the subsequent condemnation of such organs constitutes an important economic problem. A high incidence of infection in certain flocks, ranging from 31.6 per cent to 64.4 per cent, is reported.

Discovery of an early embryonal stage in the life cycle of this tapeworm, in which embryonated ova are encased in heavy fibrous capsules, is here reported.

The first indication as to the true appearance of the finished product of the ovulatory process of this worm was received in the fall of 1938. Although free ova of this cestode were observed earlier, they had not been seen in the adult worm and they had never been detected in fecal sediments. Discovery of the fact that the ova come from the worm not singly, but enclosed in egg cases, explains why single ova had not been observed routinely in the tapeworm.

Through use of a special method of dilution and decantation of fecal material obtained from autopsied or slaughtered lambs a large number of gravid, detached segments were recovered. In 266 repetitions of this procedure 540 gravid proglottids were obtained. More gravid segments were found when approximately one-fourth of the length of some worms extended through the bile duct into the duodenum. In general the numerical concentration of gravid segments was directly proportional to the degree of infection and inversely proportional to the health of the animal.

An improved method of fecal examination, reported by Bourne and Gassner, made it possible hereafter to obtain egg cases containing embryonated eggs from droppings of lambs.

The lengths of *Th. actinioides* recovered varied considerably. Two mature worms

which measured as much as 62 cm. in length and 1.4 cm. at the point of greatest width were found in the small intestine of one lamb. Immature worms measuring as little as .5 cm. were recovered from the contents of two gall bladders.

Fresh unstained preparations as well as paraffin mounts, sectioned serially, revealed embryonating ova in various stages of development in the ovarian tract of proglottids taken from the intestines. Each proglottid was observed to be hermaphroditic, containing two sets of sexual organs, which are described. The cestodes were observed to contain only one uterus. This uterus and the parauterine pouches in which embryos mature and which form the egg capsules are described.

In the fecal sediment gravid segments appeared as b l u i s h - or yellowish-white, square, cylindrical, or semilunar bodies. When slight pressure or strong light was applied to fully gravid segments, egg cases were observed emerging from the segment through an aperture in its median anterior portion. A description and measurements of egg cases and their contents as observed are given. The egg capsule seemed to afford excellent protection for the young against external influences.

Weekly examinations of gravid segments stored in physiological salt solution under refrigeration to date have shown neither aggressive nor regressive changes with the exception of a change in color of the cuticulum of some proglottids. Fecal material containing egg cases which has been incubated since January 15, 1940, to date shows no deleterious effect on either the capsules or the embryos contained therein.

Feeding experiments are under way to ascertain the possibilities of direct propagation of this parasite without an intermediate host.

Tissue Ascorbic Acid of the Goat*

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IN A PREVIOUS paper[1] the authors reported a study of the ascorbic acid content of milk and blood of goats under normal conditions, after injections of ascorbic acid, and on a vitamin C-free diet for three months. The results suggested that the vitamin C-free diet lowered the milk ascorbic acid level, but the blood ascorbic acid level did not seem to be significantly affected. It was realized that the amount of blood ascorbic acid should be related to the amount of tissue ascorbic acid; thus, the minimum amount in the blood would be affected by the amount stored or synthesized by the animal. In the continuation of the study of ascorbic acid concentration in goat's milk and blood, the ascorbic acid content of some of the tissues was also determined. The results are given in this paper.

REVIEW OF LITERATURE

By indophenol titration of trichloracetic acid extracts of goat tissues, Rudra[2] obtained the following values, expressed as mg. ascorbic acid per 100 Gm. of tissue:

Liver	*Kidney*	*Heart*	*Muscle*	*Bone Marrow*
26.3	17.6	7.7	6.6	8.8

Rudra[3] later reported finding 73.4 mg. ascorbic acid per 100 Gm. of goat liver.

Values reported for the kidneys, livers, and adrenals of some other animals have been summarized by Fixsen and Roscoe,[4] and by Richmond.[5] Most of the values given for the adrenals of normal calves, oxen, sheep, rabbits, and guinea pigs are between 100 and 200 mg. per cent. For the livers, most values fall between 20 and 70 mg. per cent. Rabbit kidney has been reported to contain from 3.5 to 25 mg. per cent.

Giroud and Leblond[6] found that when guinea pigs are completely deprived of vitamin C, the ascorbic acid content of their adrenals, testes, kidneys, etc., decrease to very small values—4 mg. per cent in the case of the adrenals—and then remain constant until death. If enough ascorbic acid is fed to raise the ascorbic acid contents of the tissues slightly above these minimum values the animals live indefinitely.

INVESTIGATION

On the basis of milk production, the twelve animals used in this work fall into two main groups, milk goats (Toggenburgs) and non-milk goats. Three of each group were fed the vitamin C-free diet, a grain mixture of four parts of white corn, three parts cottonseed meal, two parts wheat bran, and one part oats. Occasionally the grain mixture was replaced by oats alone.

The ascorbic acid content of the milk, blood, and some tissues was studied. The blood and milk samples were collected and assayed by indophenol titration as described in the previous year,[1] except that KCN was

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‡Department of Physiology and Pharmacology, Duke Medical School, Durham, N. C.

[1]Richmond, M. S., Satterfield, G. H., Grinnells, C. D., and Dann, W. J.: Ascorbic acid content of goat's milk and blood. J. Nutr., xx (1940).

[2]Rudra, M. N.: Distribution of vitamin C in different parts of common Indian foodstuffs. Biochem. J., xxx (1936), pp. 701-703.

[3]Ibid.: Studies in vitamin C. The effect of cooking and storage on the vitamin C contents of foodstuffs. Indian J. Med. Res., xxv (1939), pp. 89-93.

[4]Fixsen, Margaret A. Boas, and Roscoe, Margaret Honora: Tables of the vitamin content of human and animal foods. Nutr. Absts. and Revs., vii (1938), p. 859.

[5]Richmond, M. S.: Ascorbic acid of the goat, unpublished thesis. North Carolina State College (1940).

[6]Giroud, A., and Leblond, C. P.: Variations in the ascorbic acid content of tissues. C. R. Soc. Biol., cxviii (1935), pp. 1179-1182. Summary in Chem. Absts., xxix (1935), 4414.

replaced by NaCN as the antioxidant for blood samples.

The method used for the first two determinations of the ascorbic acid in tissues was essentially that described by Bessey and King,[7] and modified by Musulin and King.[8] The sample was thoroughly ground with acid-washed white sand and 10 to 15 cc. of an acid solution containing 8 per cent acetic acid and 4 per cent metaphosphoric acid. The mixture was centrifuged. The supernatant liquid was decanted into a volumetric flask, and the extraction repeated twice. The combined extracts were made to volume with the acid mixture. Five-cc. aliquots were titrated. This method was used for one kid of goat 7 (2/4/39) and the kid of goat M2 (3/18/39).

In order to save time and expose the tissues to less air, a modification of this method was adopted for later tissues. Centrifugation was omitted. The tissue was thoroughly ground with the extraction acid and approximately 1/50 as much acid-washed white sand as the final volume. By means of several extractions everything (sand and tissue, finely ground) was transferred to a volumetric flask, made to volume, and thoroughly mixed. Part of the mixture was filtered and 5-cc. aliquots of the filtrate were titrated.

Samples of liver and kidney usually weighed from 2 to 5 Gm. and were diluted to 100 cc. An adrenal sample consisted of a whole adrenal from a kid or one-half of an adrenal from an adult goat, and was diluted to 50 cc.

RESULTS

The results of tissue analyses are summarized in table I.

A brief summary of blood and milk results is given below.

Under Normal Conditions.—Most of the blood samples contained between 0.4 and 0.8 mg. per cent ascorbic acid. Milk showed somewhat more variation, with a usual range of 0.5 to 2.0 mg. per cent.

On the Vitamin C-free diet.—1) After the

[7]Bessey, O. A., and King, C. G.: The distribution of vitamin C in plant and animal tissues and its determination. J. Biol. Chem., cili (1939), pp. 687-698.

[8]Musulin, R. R., and King, C. G.: Metaphosphoric acid in the extraction and titration of vitamin C. J. Biol. Chem., cxvi (1936), pp. 409-413.

TABLE I—A summary of tissue analyses for ascorbic acid.

Date	Diet	Goat	Adrenal			Liver			Kidney			Remarks
			Wt. Gm.	Asc. Acid Conc. Mg. %	Asc. Acid Total Mg.	Wt. Gm.	Asc. Acid Conc. Mg. %	Asc. Acid Total Mg.	Wt. Gm.	Asc. Acid Conc. Mg. %	Asc. Acid Total Mg.	
												1-Day-Old Kids
11-22-39	Normal	Twin buck of 7	.19	88.9	.17	55.8	15.2	8.5	—	—	—	Twin died next night
1- 4-40	Normal	Twin buck of 14	.18	148.0	.26	39.8	30.6	12.2	—	—	—	Colostrum of 14 low in asc. acid
2-1-40	Normal	Twin buck of M 1	.23	111.4	.26	59.5	15.6	9.3	7.33	9.76	.72	Colostrum of M 1 high in asc. acid
												2-Day-Old Kid
1-24-40 1-25-40	Normal	Buck of 1,000	.22	74.8	.16	42.3	15.6	6.6	6.14	38.9	2.39	Unusually small, not expected to live
												8-Day-Old Kids
3-18-39	Normal	Single of M 2	.23	155.0	.36	113.0	22.5	25.3	—	—	—	
1-3-40	C-free pen	Twin buck of 794	.27	167.0	.45	125.0	25.8	32.1	—	—	—	
1-6-40	C-free pen	Twin buck of 906	.21	192.0	.41	72.1	35.9	25.9	—	—	—	
												Adults
2-29-40	Normal	M 1	1.09	94.9	1.03	602.0	31.9	192.0	44.4	13.9	6.2	Kidded 29 days ago
1-30-40	C-free pen	794	4.34	58.2	2.53	909.0	18.7	170.0	111.0	4.0	4.4	On diet 103 days Kidded 35 days ago
2-11-40	C-free pen	11	5.25	44.0	2.31	900.0	24.9	224.0	86.7	6.4	5.6	On diet 115 days*†
2-12-40		(Died)										Aborted 7 days ago
3- 2-40	C-free pen	8	.84	101.0	.85	290.0	12.9	37.4	31.0	7.3	2.3	On diet 242 days† Would not breed

*Goat 11 was given two subcutaneous injections of ascorbic acid: 1 Gm. on 2-9-40 and 0.5 Gm. on 2-10-40.

†Received 1 capsule of Smaco carotene in oil (=10,000 units vitamin A) daily from 2-1-40 to 2-15-40. (Carotene in oil supplied by S. M. A. Company of Cleveland, Ohio.)

goats were on the diet for two to three months, the blood ascorbic acid fell to about 0.4 mg. per cent. 2) During the fourth to the eighth month on the diet, the blood ascorbic acid ranged between 0.4 mg. per cent and 0.2 mg. per cent in goats M2* and 8; these two goats would not breed while on the diet. 3) The blood ascorbic acid of goat 2, the third non-milk goat on the diet,

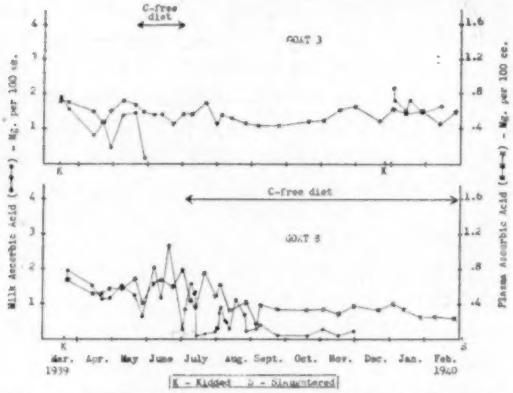

Chart 1. Effect of a vitamin C-free diet on the ascorbic acid content of blood plasma and milk.

was approximately 0.4 mg. per cent when she aborted, after 65 days on the diet; the goat had no milk; she died 29 days after abortion. 4) Goat 11 aborted after 108 days on the diet; her blood ascorbic acid was a little higher than 0.2 mg. per cent; she had almost no milk; she died seven days later. 5) Goats 794 and 906 kidded after being on the diet about 70 days; they apparently were secreting large amounts of ascorbic acid in their milk two weeks later, but only a month after kidding they were nearly dry.

The ascorbic acid contents of the blood and milk of goats 8 and M2 are compared with those of two control goats, 3 and M1, in charts 1 and 2.

DISCUSSION

The ascorbic acid content of the liver of goat M1 was 32 mg. per cent; that of goat 8 after eight months on the diet was 13 mg. per cent. These are in approximately the same ratio as the corresponding blood values, 0.6 mg. per cent and 0.24 mg. per cent. This suggests that a fall in blood ascorbic acid is accompanied by a similar decrease in general tissue ascorbic acid. Inasmuch as goats M1 and 8 were approximately the same size at the beginning of the experiment, their liver weights, 602 Gm. and 290 Gm. respectively, suggest that there was considerable atrophy in the liver of goat 8 during the experiment.

Apparently, there was some decrease in adrenal ascorbic acid of goat 8 as a result of atrophy but not from decrease in concentration. That may be a result of some special function of ascorbic acid in adrenals; King[9] has stated that there appears to be selective conservation of the vitamin

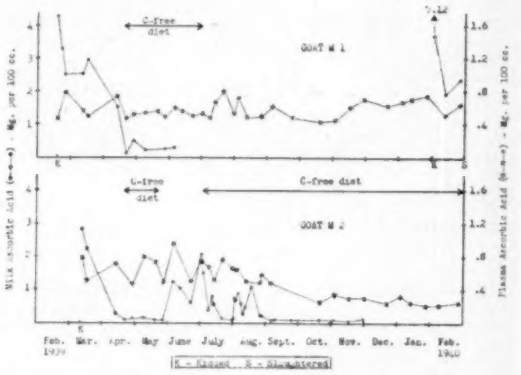

Chart 2. Effect of a vitamin C-free diet on the ascorbic acid content of blood plasma and milk.

in the more vital tissues during a period of general body depletion. It should be remembered, though, that the total quantity in the adrenals probably represents a very small fraction of the total normally present in the body.

Richmond[5] has estimated the total quantities of ascorbic acid lost by a goat through milk, urine, and kids, that taken in through food, and that present in a goat's body. If first grade alfalfa hay contains 1.88 mg. per cent ascorbic acid as reported by Holmes, Tripp, Woelffer, and Satterfield,[10] then just

*M2, 8, 2, M1, and 3 were non-milk goats; 11, 794, and 906 were milk goats.

[9]King, C. G.: The physiology of vitamin C. The Vitamins, American Medical Association, Chicago, Illinois, (1939), pp. 331-338.

[10]Holmes, A. D., Tripp, F., Woelffer, E. A., and Satterfield, G. H.: A study of breed and seasonal variation in the ascorbic acid content of certified milk from Guernseys and Holsteins, J. Nutr., xvii (1939), pp. 187-198.

the hay of a recommended daily ration for a goat may contain 25 mg. ascorbic acid. This more than balances the average amount estimated to be lost by the goat in milk, urine, and kids.

It was also estimated that goat M1 may have contained 1290 mg. ascorbic acid at the time of slaughter. If it is assumed that the decrease in tissue concentration is similar to that in blood concentration, then 8 lost approximately one-third of her ascorbic acid in about 55 days on the diet, and another one-third in the next 200 days. There was additional loss from decrease in body weight. It is conceivable that all the ascorbic acid lost by goat 8 in milk and urine during eight months on the vitamin C-free diet was furnished by tissue depletion.

It is interesting to note that goat 1000 had the largest milk production and maintained a relatively high ascorbic acid concentration in milk, and she had an exceptionally small weak kid that apparently was low in ascorbic acid.

On the other hand, goats 906 and 794 both had twins and those of goat 794 were exceptionally large. These two goats had the lowest milk production, and the milk of goat 794 was fairly consistently low in ascorbic acid.

All goats used in this investigation were tested for *Brucella abortus* infection and found negative.

There have been several indications in the literature that vitamin C deficiency may be responsible for reproductive failures. Hoseman[11] suggests that partial vitamin C deficiency may be a contributory cause of spontaneous abortions in humans. Kramer, Harman, and Brill[12] reported that vitamin C deficiency produces degeneration of corpora lutea and failure of normal gestation in guinea pigs. Biskind and Glick[13] suggested that vitamin C is necessary for the normal functioning of corpora lutea of cows.

It may be wondered if breeding problems in this investigation resulted from a deficiency of vitamin C. It was impossible to breed five milk goats—11, 10, 1000, 794 and 906—on a normal diet during the season 1938-39. Whether that was a result of depletion of tissue ascorbic acid by lactation is a question.

Turner[14] stated that the goat is a seasonal breeder, with an anestrus period extending from March until August. This may possibly be related to the storage of ascorbic acid from summer pasturage, and a loss from reproduction and lactation during the winter. In view of the work of Hawley, Daggs, and Stephens,[15] the fact that goat urine is normally more alkaline than that of humans suggests that tissue storage of ascorbic acid may be normally greater in goats than in humans.

All of the goats were injected with gonadin serum (Cutter) in August 1939. Even then goats M2 and 8 did not breed, or if they did, they resorbed the fetuses. However, unless further evidence is obtained otherwise, these two breeding failures probably should be ascribed to vitamin A deficiency. The vitamin C-free diet was not planned for a longtime experiment. It was deficient in calcium, high in phosphorus, and deficient in vitamins A and D. These defects may have caused the infections observed, and the infections may have been the cause, rather than the effect, of the ascorbic acid deficiency.

According to the literature there seems to be some relation between ascorbic acid and lipid metabolism. In this investigation it was observed that there was much more deposited fat in the peritoneal cavities of

[11]Hoseman, H. (with Athanassiu, G.): Possibility that Vitamin C deficiency is a cause of habitual abortion. Zentralbl, Gynakol, lxiii (1939), pp. 1784-1792. Summary in Nutr. Absts. and Revs., ix (1940), p. 730.

[12]Kramer, M. M., Harman, M. T., and Brill, A. K.: Disturbances of reproduction and ovarian changes in the guinea pig in relation to vitamin C deficiency. Am. J. Physiol., cvi (1933), pp. 611-622.

[13]Biskind, G. R., and Glick, D.: Studies in histochemistry, V. The vitamin C concentration of the corpus luteum with reference to the stage of the estrous cycle and pregnancy. J. Biol. Chem., cxiii (1936), pp. 27-34.

[14]Turner, C. W.: Seasonal variation in the birth rate of the milking goat in the United States. J. Dairy Sci., xviv (1936), pp. 619-622.

[15]Hawley, E. E., Daggs, R. G., and Stephens, D. J.: The effect of the administration of acid and alkaline salts upon the ascorbic acid content of guinea pig tissue. J. Nutr., xiv (1937), pp. 1-8.

the experimental animals (8, 794, and 11) than in that of the control animal (M1), even though the former goats had apparently lost weight. This suggests that if the ascorbic acid content of the body is subnormal there may be a tendency for food to be stored as fat rather than as liver and muscle glycogen. Attention is called, here, to the report of McHenry and Sheppard[16] and that of Gajdos.[17]

On the average the values determined for ascorbic acid in goat's milk in this investigation are somewhat lower than those reported for cow's milk. This may be normal or it may result from a suboptimal supply of ascorbic acid in the diet that was considered "normal" in this investigation. It is possible that workers who have reported that the ration does not affect the milk ascorbic acid were using a control diet that furnished enough ascorbic acid to maintain tissue saturation, or did not continue the experiment long enough to obtain tissue depletion.

Madsen, McCay, and Maynard[18] also obtained some evidence that goats need ascorbic acid in their diet.

SUMMARY AND CONCLUSIONS

1) Half of a group of twelve goats were fed a vitamin C-free diet; the ascorbic acid contents of the milk, the blood, and some tissues were determined by indophenol titration.

2) The diet consisted of a grain ration only. Consequently, it was deficient in other factors as well as in ascorbic acid.

3) The diet was not adequate for normal reproduction and lactation. The two goats already on the diet would not breed. The other four were placed on the diet during gestation; two aborted—one after two months, the other after three and a half months; all four were subnormal in milk production.

4) After the goats were on the diet for two to three months, the blood ascorbic acid was about 0.4 per cent or approximately two-thirds the normal average concentration. There was also a decrease in milk production.

5) In the two non-milk goats which were not bred, the blood ascorbic acid concentration decreased approximately one-third in 55 days, and another one-third in the next 200 days on the diet.

6) Tissue analyses indicated that the decrease in blood ascorbic acid concentration was accomplished by a similar decrease in liver ascorbic acid concentration but not in that of the adrenal glands. Apparently, there was a decrease in total ascorbic acid of both the adrenal glands and the liver as a result of atrophy.

7) There was more deposited fat in the peritoneal cavities of the experimental animals than in that of a control animal, even though the former had apparently lost weight.

8) Estimations have been made which show that the intake of ascorbic acid in a normal ration may be as much as the goat loses in milk, urine, and kids. Also, the amount excreted by goats on the vitamin C-free diet could have come from tissue stores.

[16]McHenry, E. W., and Sheppard, M.: Physiological properties of ascorbic acid. J. Nutr., xvii (1939), p. 20.

[17]Gajdos, A.: Effect of ascorbic acid on the blood serum lipase. C. R. Soc. Biol., cxxxi (1939), pp. 59-61.

[18]Madsen, L. L., McCay, C. M., and Maynard, L. A.: Synthetic diets for herbivora with special reference to the toxicity of cod liver oil. Cornell Agr. Exp. Sta. Memoir, No. 178 (1935), 53 pp.

Some Observations on Nephritis in Horses*

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DURING the past two years, the department of animal pathology was able to obtain the kidneys from 38 horses immediately after death. Realizing that there has been very little published on the histology and micropathology of the horse's kidney, a study was made of these cases.

Six of the cases were obtained from the Michigan State Department of Health Laboratories where the animals had been on serum production and hyperimmunized against types I and II pneumococcus, diphtheria toxin, etc. At the time of death, two of the animals had developed an anemia. Thirty of the cases were aged animals purchased by the department of surgery and medicine and used for surgical exercises. Most of these animals were in fairly good condition. Two showed a severe laminitis, one had melanotic tumors and another a tumor of the eye. The ages of these animals ranged from 7 to 27 years with an approximate average of 16½ years. The remaining animals were 6 months and 1 year old, respectively.

The usual gross lesions consisted of some hyperemia, occasional small cysts in the cortex and medulla, and a few circumscribed white foci, shrunken or depressed, were occasionally present.

Four of the cases that appeared to be the most normal were studied histologically by both the technic of maceration and fixed and stained methods. The approximate average diameter of the different segments of the nephron are recorded in table I. In general, the histological characteristics of the horse's kidney are similar to those of the cow. However, the distal tubule is very short and has no apparent convolutions (fig. 1).

TABLE I.*

		Renal Corpuscle			Prox. Con. Tubule			Term. Port. of Con. Tubule			Distal Con. Tubule			Descending Limb of Henle			Ascending Limb of Henle		
Aut.	Age	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
1549	10 yrs.																		
Macerated		364	272	325	111	62	82	72	42	55	78	56	70	29	13	24	41	31	37
Stained		281	218	247	70	45	58	45	31	38	64	38	49	25	13	20	39	28	33
1548	15-17 yrs.																		
Macerated		272	163	238	87	58	71	58	41	50	76	54	65	27	14	19	37	27	32
Stained		257	190	232	68	41	48	48	34	40	64	41	51	30	13	21	40	29	34
1522	16 yrs.																		
Macerated		392	200	311	78	64	71	73	29	50	77	64	72	27	13	19	41	28	34
Stained		290	218	262	92	46	60	43	26	35	68	40	50	36	19	28	53	33	40
1523	17 yrs.																		
Macerated		281	183	221	89	42	67	63	38	49	73	40	55	27	11	17	38	27	32
Stained		218	145	177	63	34	49	44	27	37	63	35	48	29	14	23	38	27	34

*The data shown are the result of measurements of 25 units for each animal given. The measurements were made on material obtained by macerating formalin-fixed tissue in hydrochloric acid and also paraffin, hematoxylin-eosin stained sections of Zenker's fixed tissue.

The abbreviations, max., min., prox., con., term., and port., indicate maximum, minimum, proximal, convoluted, terminal, and portion, respectively. The numerals indicate diameter in microns.

Microscopically, eight of the cases showed some degree of nephritis. The lesions

*From the Animal Pathology Section, Michigan Agricultural Experiment Station; journal article No. 426(n.s.) of the Michigan Agricultural Experiment Station.

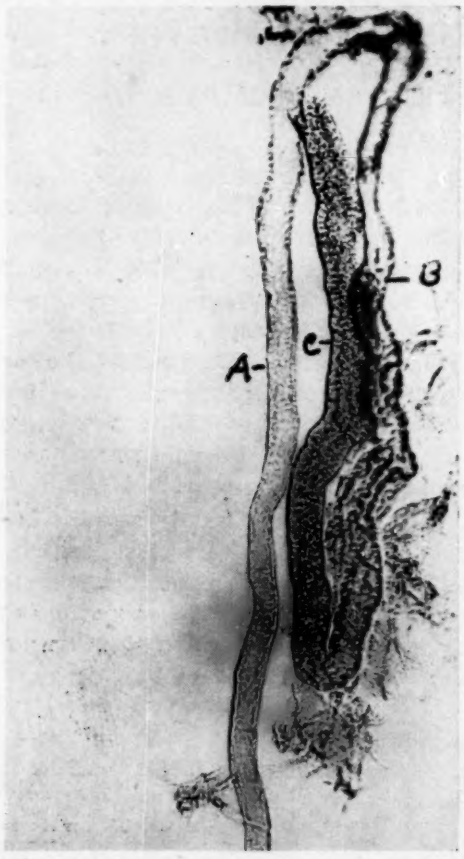


Fig. 1. Macerated specimen showing A — ascending limb of Henle; B—distal tubule; C—collecting tubule. Observe the shortness of the distal tubule and lack of convolutions (x 100).

were usually focal in distribution and primarily involved the interstitial connective tissue. The lesions usually begin as perivascular accumulations of lymphocytes and macrophages which infiltrate the interstitial connective tissue (figs. 2-5) followed later by proliferation of fibroblasts and fibrosis, leading to atrophy and ultimate loss of the tubules (fig. 6).

In general, the renal corpuscles showed little change other than a slight thickening of Bowman's capsule with connective tissue (fig. 7). Occasionally, the glomeruli showed thickening of the tufts, adhesions to the wall of the capsule of Bowman, and

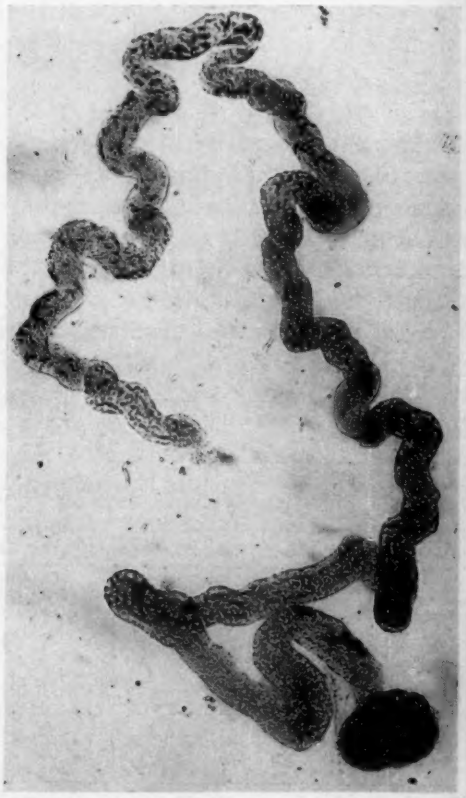
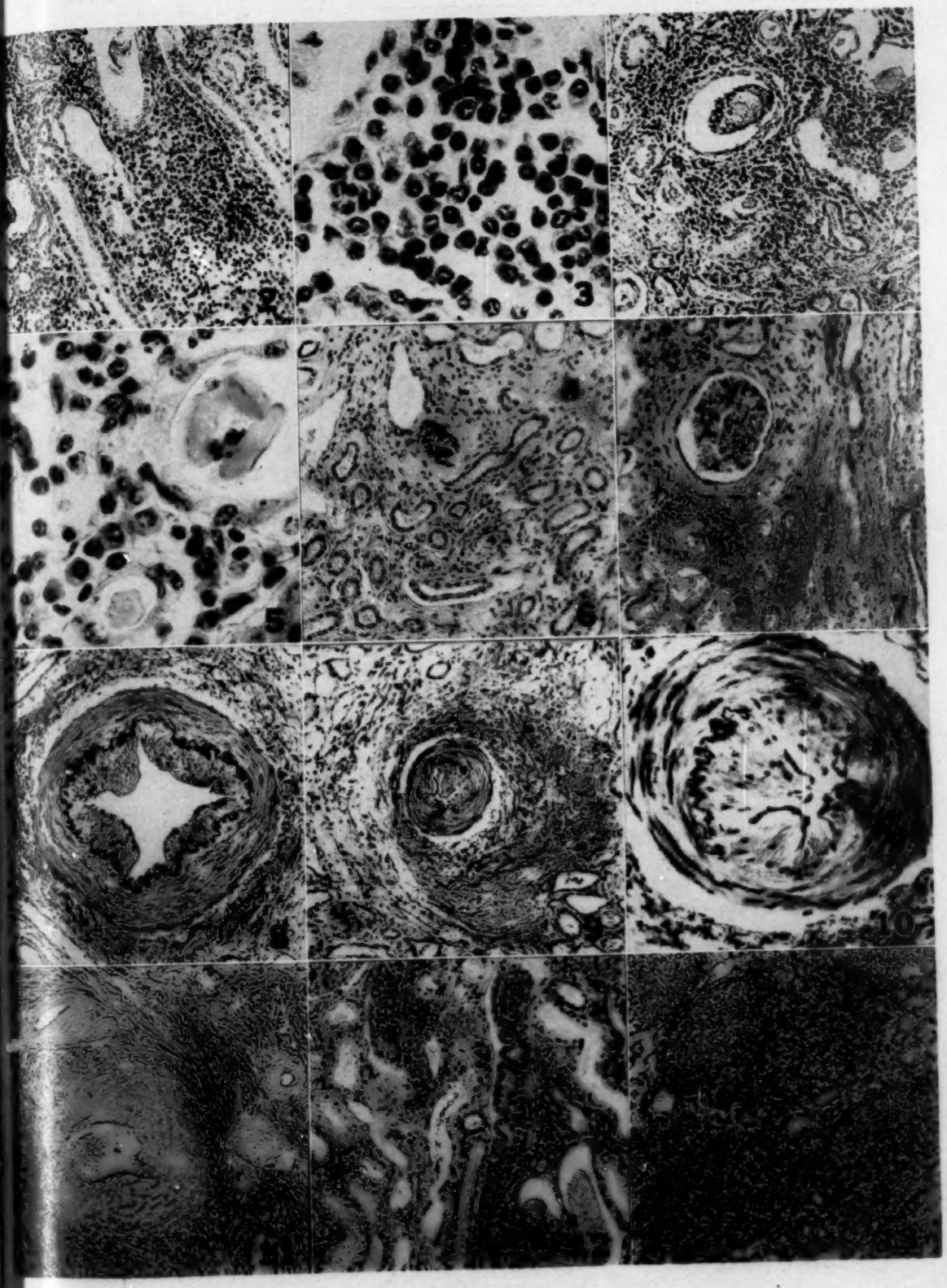


Fig. 14. Macerated specimen, showing renal corpuscle and part of the proximal convoluted tubule (x 66).

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE

Fig. 2. Section showing focal accumulations of macrophages and lymphocytes in the cortex (x 117). Fig. 3. Same as figure 2, only higher magnification (x 660). Fig. 4. Accumulations of lymphocytes and macrophages leading to atrophy and ultimate loss of the tubules (x 117). Fig. 5. Same as figure 4, only higher magnification. Observe hyaline casts in degenerate tubules (x 660). Fig. 6. Atrophy and loss of tubules due to fibrosis (x 117). Fig. 7. Thickening of Bowman's capsule with connective tissue and perivascular accumulations of lymphocytes around a small arteriole (x 117). Fig. 8. Thickening of the intima in an arciform artery (x 117). Fig. 9. Section of interlobular artery showing almost complete occlusion of the vessel due to subendothelial proliferation (x 117). Fig. 10. Same as figure 9, only higher magnification. Observe the fragmentation of internal elastic membrane in some parts and its absence in other parts (x 521). Fig. 11. Hyalinization of walls of smaller blood vessels (x 117). Fig. 12. Folding of the epithelium of the ascending limb of Henle (x 117). Fig. 13. Accumulations of red blood cells in the collecting tubules (x 117).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loss of some epithelial cells. A few scattered renal corpuscles showed a partial to complete hyalinization.

The most interesting lesions in our study were those found in connection with the blood vessels. In 23 of the cases, the blood vessels ranging from the small interlobulars to the large arciforms showed changes, especially the ones around 100μ to 200μ in diameter. The intima showed thickening in many cases due primarily to growth of the subendothelial connective tissue (fig. 8). In one case, several of the arterial lumens were practically occluded. In some instances, the internal elastic membranes were fragmented and reduplicated (figs. 9 and 10). The walls of some of the smaller arterioles were hyalinized (fig. 11). In one or two cases there was medial degeneration and atrophy of the smaller arteries. In all of these cases, very little kidney change could be seen as a result of the arteriolar changes.

Some of the cases showed cloudy swelling, pyknosis, and desquamation of the epithelium of the tubules. Occasionally, a peculiar folding of the epithelium of the ascending loop of Henle had taken place and practically occluded the lumen (fig. 12). Here and there, casts and cystic tubules were observed. One case showed an extensive hemorrhagic condition in which the collecting tubules were distended with blood (fig. 13).

Report on the Relationship of Thyroid Activity to Periodic Ophthalmia*

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TAGLIAVINI[1] asserted that periodic ophthalmia is due to thyroid hypofunction. In over 50 cases he reported the thyroid glands were in a state of hypofunction (dysendocrinia) as determined by a histophysiological examination.

A histophysiological examination was conducted on the thyroid glands of 21 horses with normal eyes and on the thyroid glands of seven horses reported as being affected with periodic ophthalmia on clinical examination. The criteria used to evaluate the activity of these glands were similar to those expounded by Tagliavini, namely, height and activity of the follicular epithelium, and consistency and staining reaction of the colloid.

Additional information concerning the histophysiology of the thyroid gland was obtained from articles by Hellwig[2], Hewer[3], and Williamson and Pearse.[4, 5, 6]

TECHNIC

All material was sectioned in triplicate and stained with hematoxylin and eosin, Mallory's phosphotungstic acid hematoxylin and Mallory's aniline blue—orange G stain without the acid fuchsin according to the method of Hewer.[3]

All phases of secretory activity were observed in the thyroid glands of the 21 horses with normal eyes. The histophysiological picture varied from one of intense

*Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture.

[1]Tagliavini, A.: Über die rezidivierende uveitis des pferdes. Arch. f. wiss. u. prakt. tierheilk., lxxiii (1938), pp. 66-70.

[2]Hellwig, C. A.: Histology of thyroid in exophthalmic goiter and hyperthyroidism. Arch. Path., xxviii (1939), pp. 870-904.

[3]Hewer, E. E.: Activity of the thyroid gland in relation to the staining reactions of the colloid. J. Path. & Bact., xxx (1927), pp. 621-625.

[4]Williamson, G. S., and Pearse, I. H.: A system of tubules in secreting epithelia. J. Anat., lvii (1923), pp. 193-198.

[5]Ibid.: Thyroid apparatus in man. Lancet, ccv (Dec. 22, 1923), pp. 1337-1340.

[6]Ibid.: Applied anatomy and physiology of the thyroid apparatus. Brit. J. Surg., xiii (1926), pp. 466-496.

activity to one of relative quiescence. The active glands were characterized by small amounts of foamy alkaline colloid stored in follicles lined with columnar epithelium. The inactive glands were characterized by a large amount of compact colloid with a tendency to acid reaction stored in dilated follicles lined with flattened epithelium. Many intermediate stages of activity were observed, and in most glands different types of follicles representing various stages of secretory activity were observed in the same section. A more or less arbitrary evaluation of the activity of any particular gland was attempted by determination of the type of follicle which predominated.

The thyroid glands of the seven horses affected with periodic ophthalmia varied from a moderate to a pronounced state of secretory activity. The inactive, sluggish type of gland described by Tagliavini was not observed. These findings can not be regarded as conclusive because only a small amount of material was brought under observation and because most of the horses used in the experiment died as the result of an infectious disease or were killed during the terminal stage of such a disease. (It is known that toxic conditions may produce functional activity in the thyroid gland.) However, one of the most active glands found in the group of periodic ophthalmia horses (case 1127) was obtained from a horse that had been slaughtered in good health.

Marine[7] states that, "It is difficult to define the normal elements of the thyroid because of the extreme liability of this tissue. Variations in size and structure not only may occur from day to day, but there are variations dependent upon season, climate, geographical location, age, sex, food and state of nutrition, which have to be taken into consideration." In the case of a gland that is so sensitive to environmental changes, any effort to ascribe pathogenic significance to any particular functional phase of that gland would require a large amount of supporting evidence.

Regarding the treatment of thyroid hypofunction with thyroidin, the following quotation is extracted from Hellwig[2]: "Administration of thyroid extract or of thyroxin decreases the activity of the thyroid as demonstrated by Cameron and Carmichael, Gray and Rabinovitch, Kuchinsky and Eggert. The acinar epithelium becomes flat and the cell borders indistinct, and the cytoplasm contains only few fuchsinophilic granules. Andersson's vacuoles and intracellular colloid droplets are absent. The colloid is stored in the follicle as concentrated substance with few or no vacuoles. There is also a decrease or complete absence of mitotic figures in the thyroid cells. The opposite effect is observed after injection of an extract of the anterior lobe of the pituitary gland containing the thyrotropic factor, which apparently is produced by the acidophile cells. Its effect on the thyroid has been well established. It is the most dependable and fastest means of producing thyroid hyperfunction."

SUMMARY

Histophysiological examination of the thyroid glands of seven horses clinically affected with periodic ophthalmia failed to reveal evidence of hypofunction. The thyroid glands of 21 horses with normal eyes revealed all phases of secretory function, varying from intense activity to relative quiescence. These limited observations are not in accord with those of Tagliavini who associates thyroid hypofunction with periodic ophthalmia.

[7]Marine, D.: The thyroid, parathyroids and thymus. Cited in Cowdry, E. V.: Special Cytology I, pp. 551-619 (P. B. Hoeber, New York, 1928).

The Relation of Brucellosis to Periodic Ophthalmia in Equidae*

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In May 1939, Burky and associates[1] announced their theory that Brucella infection is responsible for periodic ophthalmia in horses. With a view to confirming or refuting this claim, experimental studies were initiated at our research laboratory here at Front Royal. Efforts in this direction have been threefold: 1) agglutination tests, using *Brucella abortus* antigen to test the sera of normal and ophthalmia-affected horses; 2) complete cultural examinations of animals destroyed with the disease; and 3) gross and histopathological studies of all organs of cases autopsied.

Serological Studies

It is known that Equidae do develop agglutinins to Brucella organisms in positive cases of infection.[2] These are frequently encountered in chronic necrotic infections of the thoracic supraspinous and atlantal bursae (fistula of withers and poll evil).[3, 4] Organisms of the genus Brucella are known to infect horses, although it is not an especially common infection.[5]

We have completed agglutination tests on the sera of normal control animals and on that of horses affected with periodic ophthalmia. Control sera were collected from animals of different ages at this remount depot. Each control animal was examined ophthalmologically and animals showing ocular lesions of any kind were excluded from the series. Accurate clinical records are kept on all military animals. No control animal had any history or lesions of periodic ophthalmia.

Sera from cases of periodic ophthalmia were collected from animals at this depot and from army animals at other stations located in Vermont, Illinois, Oklahoma, Virginia, Maryland, Kansas, Georgia, Colorado, and Nebraska. Tests were repeated on many animals as they passed through the different stages of the disease, but only one test from each animal is included in table I. The highest titre reached is the one which is recorded in table I.

It will be noted in table I, that agglutinations occurred in very few of the sera in dilutions that are high enough to be of positive diagnostic significance (1:800 or higher). Young horses (1 year or under) appear to exhibit relatively fewer agglutinins than older horses. Aside from the higher percentage of completely negative reactions in the control sera, which may be explained on the basis of a larger number of control tests in the younger age group, the reactions among normal sera are nearly identical with those of the ophthalmia sera.

Burky observed that the agglutination titre of the sera of some horses may rise from 1:40 in the quiescent stage to 1:80 in the acute stage of periodic ophthalmia. Upon cessation of acute symptoms the titre returned to 1:40. We have observed this same phenomenon in a few cases. In a great majority of cases under our observation, however, no change in the agglutination titre could be correlated with the appearance of acute symptoms. Further, the sera of many horses with typical acute ocular symptoms are entirely negative in all dilutions.

In order to facilitate the study of the pathology of periodic ophthalmia, cases of the disease are divided into three classifications based upon their clinical stage. These are: acute, quiescent, and old. An acute case is one in which the active re-

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[1]Burky, E. L., Thompson, R. R., and Zepp, Helen M.: The role of Brucella in human and ocular diseases with special reference to periodic ophthalmia in horses. Amer. J. Ophth. xxii (1939), pp. 1210-1217.

[2]Carpenter, Chas. M., and Boak, Ruth A.: The significance of the horse in brucellosis. J. Bact. xxxiii (1937), p. 40.

[3]Deem, A. W.: Brucella abortus in horses. J. Bact. xxxiii (1937), p. 40.

[4]Stone, W. S.: Brucellosis in horses. Cornell Vet. xxviii (1938), p. 91.

[5]Huddleson, I. Forest: Brucellosis in Man and Animals (The Commonwealth Fund, New York, 1939).

TABLE I—Agglutination titre of horses of various ages to Br. abortus.

CONTROL SERA

TITRE	1 YR. & UNDER	2 YRS.	3 YRS.	4 YRS.	OVER 4 YRS.	TOTAL	PERCENTAGE
NEG. ALL DIL.	34	12	4	2	9	61	32.6
1:20	18	6	2	1	28	55	29.4
1:40	5	5	3	1	38	52	27.8
1:80	1	0	0	0	16	17	9.1
1:160	1	0	0	0	1	2	1.1
1:320	0	0	0	0	0	0	0.0
Total	59	23	9	4	92	187	100.0

OPHTHALMIA SERA

NEG. ALL DIL.	0	1	0	0	17	18	18.9
1:20	1	3	1	0	28	33	34.7
1:40	2	2	5	3	22	34	35.8
1:80	0	2	0	0	5	7	7.5
1:160	0	0	0	0	3	3	3.1
1:320	0	0	0	0	0	0	0.0
Total	3	8	6	3	75	95	100.0

current inflammatory symptoms are present in the eye. In a quiescent case the acute symptoms are in abatement, but may reappear at any time. An old case is one in which the acute symptoms have entirely ceased to appear. Of course, it is very difficult to predict when the active disease has finally ceased, but any case that has shown no acute symptoms for over a year and presents the typical chronic degenerative changes, we arbitrarily classify as old.

Table II shows the stage of the disease in the 95 cases of periodic ophthalmia in this series. It will be noted that 29 were acute, 35 quiescent, and 31 old. These numbers are roughly comparable, since they each approximate 30. It may readily be seen that the agglutination titres in the different stages of the disease are approximately equal. It is especially noticeable that the agglutination titre of the acute cases is not markedly higher than that of quiescent and old cases.

BACTERIOLOGICAL STUDIES

Reports in the literature concerning the bacteriological aspects of periodic ophthalmia have usually been limited to studies of material from the affected ocular structures (contents of bulb, conjunctiva, optic nerve, etc.). No one organism has been repeatedly isolated. Since it is possible that an organism could localize in some other part of the body and still produce changes in the eye through its toxin, we have made complete cultural examinations of various organs of the body of most of the cases that we have autopsied. The following were studied bacteriologically: liver, spleen, genitalia, mammary gland, various lymph glands and adrenals.

Cultures were made in normal atmos-

TABLE II—Agglutination to Br. abortus of sera from horses in various stages of periodic ophthalmia.

TITRE	STAGE OF THE DISEASE			TOTALS (ALSO SEE TABLE I)
	ACUTE	QUIESCENT	OLD	
NEG. ALL DIL.	*6	3	9	18
1:20	10	12	11	33
1:40	11	15	8	34
1:80	2	2	3	7
1:160	0	3	0	3
1:320	0	0	0	0
Total	29	35	31	95

*Number of sera tested.

phere and in 10 per cent carbon dioxide on blood agar, chocolate agar, liver infusion agar (plus gentian violet), nutrient agar, and dextrose broth. Rather large pieces of the organ to be cultured were removed at the time of autopsy with sterile forceps and scissors and placed in sterile petri dishes for transportation to the laboratory. Cultures were made within an hour or two. The surface of the organ was seared with a hot spatula and small cubes (1 x 1 cm.) of tissue were cut out with sterile scissors and placed with sterile forceps into another sterile petri dish. Care was taken to include any lesions that might be present. The pieces were then cut into several smaller sections with the scissors. A hot platinum loop was then touched to the small bit of tissue. The heat caused the loop to adhere to the tissue which was then transferred to the culture tubes and thoroughly rubbed over the surface of the media. Incubation was carried out in atmosphere and 10 per cent carbon dioxide at 37.5° C. until growth appeared. If no growth appeared, the cultures were incubated for a minimum of two weeks (usually longer) before being called negative.

Thirty-two cases of periodic ophthalmia have been autopsied to date. Of these, 27 were cultured. In no case were organisms of the genus Brucella encountered, although the technic used is believed to be suitable for their isolation. In 13 cases no organisms of any kind were isolated from the cultures made. From 14 cases, a pleomorphic, ovoid, Gram-positive rod was isolated. In 11 of these cases this organism was found in pure culture. In three cases, *Escherichia coli* also appeared in the original cultures (in these three cases over an hour elapsed from the time of death to the time of the autopsy). In all cultures, the Gram-positive ovoid rod produced numerous colonies on the original media. This organism may be briefly described as a medium-sized, Gram-positive rod with rounded ends but having a tendency to be pleomorphic as coccoid forms are sometimes seen, and at other times swollen forms appear. The cells are usually paired but often form chains. It is motile, nonsporulating, and non-acid-fast. Chromogenic granules are occasionally seen in cells from freshly isolated cultures. It grows slowly; best growth occurring on blood agar or dextrose broth. It produces small, white, circular, umbonate colonies about 2 to 3 mm. in diameter on blood agar in three to four days. It produces acid but no gas from dextrose, salicin, and usually raffinose. No fermentation occurs in sorbitol, lactose, or xylose broth.

Intravenous inoculation into rabbits produces a marked monocytosis in the rabbits. No other symptoms were observed. Intravenous inoculation into one horse produced no reaction. Tentatively, we place this or-

ganism in the genus Listerella. Its significance in periodic ophthalmia, if any, has not been established.

ANIMAL INOCULATIONS

Material from the livers, spleens, and genitalia of six cases of periodic ophthalmia was emulsified and inoculated into guinea pigs intraperitoneally. After six weeks the guinea pigs were destroyed and autopsied. In no case were lesions of brucellosis observed in any of these animals. Attempts to isolate organisms of the genus Brucella from these guinea pigs were unsuccessful. Complete pathological studies are being made of tissues from the cases autopsied. Results of these studies will be included in a future report.

SUMMARY AND CONCLUSIONS

The results of agglutination tests with sera from 187 normal horses and 95 horses with periodic ophthalmia are reported. Complete autopsies have been conducted on cases of periodic ophthalmia, destroyed in various stages of the disease. Out of 32 cases autopsied, complete bacteriological studies were made of 27. Gross and histopathological studies were also made, and are to be included in a future report.

The experimental work outlined is being continued. Although the series of cases studied is still relatively small, the following may be stated as indicated by the work in progress: cultural and serological tests indicate that Brucella organisms are not constantly present in periodic ophthalmia. Organisms of the genus Listerella have been isolated from various organs in 14 out of 27 periodic ophthalmia cases studied culturally. Their significance has not been established.

Preliminary Studies on the Effects of Diet upon Internal Parasites in Horses*

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RECENTLY unofficial statements have been rather widely disseminated to the effect that prunes fed to horses act as an anthelmintic and that parasitic worms are found abundantly in the feces of such animals. In view of these statements and particularly because of the fact that nutrition plays an important rôle in determining the susceptibility to and pathogenesis of parasitic invasion, advantage was taken of the opportunity to conduct preliminary investigations upon the effects of a prune diet and also upon the effects of a vitamin A-deficient diet on strongyle and *Parascaris equorum* infections in horses.

METHODS

All horses used in this investigation were purebred Percherons, bred and reared in the regular Stud of the University of California.

In the prune feeding tests six 3-year-old animals were used. Previous to the investigation they had been kept on an irrigated pasture. The diet to which they were subjected consisted of 16 to 17 lb. of prunes, a little meadow hay deficient in calcium, 3 lb. of barley, ½ lb. of cottonseed meal and a little straw with molasses.

In the vitamin A-deficient tests six animals, five of which were 2 years old and one, 4 years old, were used. These animals were removed from the irrigated pasture and kept, for several months before the parasitological investigations were initiated and during the course of the investigations, on the following diets. Animals No. 5 and No. 6 were given a ration composed of 30 lb. of barley, 30 lb. of oats, 30 lb. of wheat bran, 5 lb. of linseed meal, 5 lb. of cottonseed meal and no roughage. Animal H_2 (4-year-old) was given the same ration plus 1½ lb. of calcium carbonate. Animals No. 7 and No. 8 were given a ration consisting of 55 lb. of beet pulp, 10 lb. of barley, 10 lb. of oats, 10 lb. of wheat bran, 10 lb. of linseed meal, 5 lb. of cottonseed meal and no roughage. Animal No. 10 was given the same ration plus 1½ lb. of calcium carbonate.

The eight animals composing the control group were chosen at random from the University Stud and were of various ages. Two were 1 year old, two were 7 years old, one was 8 years old, one was 10 years old, one was 14 years old and one was 15 years old. All of these animals had been kept under essentially the same conditions of management, spending part of the time on irrigated pastures and part of the time in the barn. During the course of investigations these control animals were managed in the same way as they had been, but the other two groups were kept in separate corrals.

The corral in which the prune-fed horses were kept was dry, but the corral in which the vitamin A-deficient horses were kept had a wet area and considerable soft mud in certain portions.

Three egg counts were made from fecal samples taken from the prune-fed animals before the prune diet was initiated in order to determine the initial magnitude of infection. The initial infection in the vitamin A-deficient horses was not determined before they were subjected to the deficient diet. It is reasonable to suppose that the infection would be similar to that of the control group because they were selected out of this group at the time they were placed on the vitamin A-deficient diet.

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After the prune diet was initiated egg counts were made weekly for seven weeks for each of the horses in this group. Egg counts, totaling 5, were made every two weeks after the second count for each of the horses in the vitamin A-deficient and control groups except as indicated in the following tables. In making the egg counts the Caldwell Method as modified by Stewart, Miller and Douglas[1] was employed. Two counts were made from each sample and averaged, which permitted the quantitative detection of eggs in as low a concentration as 50 eggs per gram of feces.

Only strongyle and *P. equorum* eggs were found in the fecal samples examined.

DISCUSSION

Table I shows the results of the egg counts made for the prune-fed horses on the basis of number of eggs per gram of feces.

No Parascaris eggs were found in the feces from the prune-fed animals, with the exception of in the last count made for Horse No. 100 where 100 *P. equorum* eggs per gram of feces were discovered; these Parascaris eggs are not recorded in table I. Therefore, all of the eggs recorded in table I are strongyle eggs.

TABLE I—Eggs per gram of feces in prune-fed horses.

DATE	HORSE NO.					
	100	101	102	103	104	105
1-20-39*	450	250	2,100	750	100	1,650
1-26-39*	750	450	1,200	500	50	1,450
2- 1-39*	1,000	1,850	1,450	450	250	1,350
3- 1-39	1,300	4,450	1,850	1,250	1,050	3,850
3- 8-39	2,300	800	3,500	600	750	2,450
3-15-39	4,250	650	1,700	2,400	450	2,650
3-21-39	2,100	850	800	550	250	1,350
3-28-39	1,750	1,250	4,300	1,050	350	3,650
4- 5-39	2,900	1,250	3,000	1,250	100	3,150
4-11-39	4,250	1,800	4,900	1,950	400	3,950

*Egg counts made before prune diet was initiated.

From table I it is readily seen that in all animals, with the possible exception of Horse No. 104 which had but a very light initial infection of strongyles, the number of eggs per gram of feces markedly increased after the initiation of the prune diet. Unfortunately the data do not permit statistical analysis to determine the actual insignificance of these differences but in the opinion of the authors these differences are great enough and sufficiently consistent to warrant considering them mathematically significant.

Table II shows the results of the egg counts made for the horses on a vitamin A-deficient diet on the basis of number of eggs per gram of feces.

It is seen from table II that the vitamin A-deficient horses were much less heavily infected with strongyles than were the prune-fed horses and that the former were from lightly to fairly heavily infected with *P. equorum* while eggs of this parasite were found only once in one of the latter animals. The inference might be drawn from these data that a vitamin A-deficient diet is unfavorable for strongyles. However, these horses had not been placed on a pasture for about a year and most of the parasites acquired at that time would have matured and died. On the other hand, the vitamin A-deficient animals practiced mud-eating and could thereby have continually reinfected themselves.

These same data also possibly indicate

[1] Stewart, M. A., Miller, R. F. and Douglas, J. R.: Resistance of sheep of different breeds to infestation by *Ostertagia circumcincta*. J. Agri. Res., lv (1937), pp. 923-930.

TABLE II—Eggs per gram of feces in vitamin A-deficient horses.

DATE	HORSE NO.					
	H_2	10	7	8	5	6
2- 7-39	550p 0s	5500p 200s	100p 150s	750p 200s	0p 100s	0p 0s
3- 1-39	350p 100s	4100p 100s	200p 0s	950p 350s	50p 100s	350p 100s
3-15-39	50p 50s	3300p 0s	250p 0s	800p 50s	150p 50s	Died
3-27-39	Died	2450p 150s	0p 0s	500p 50s	250p 0s	.....
4-11-39	.....	4100p 450s	0p 0s	350p 100s	250p 50s	.....

p = *P. equorum.*
s = Strongyle eggs.

that a vitamin A deficiency lowers equine resistance to *P. equorum* infection. This is not the first suggestion that avitaminosis may lower resistance to helminthic infections. Hiraishi[2] has shown that pigs fed on a vitamin A-deficient diet lost their resistance against the porcine strain of *Ascaris lumbricoides* and also against the human strain of the parasite which ordinarily fails to mature in swine. On the other hand, Clapham[3] was unable to show from experiments on pigs that the vitamin A content of the diet had any appreciable effect on the course of infection with *A. lumbricoides.* This same author (Clapham[4]), however, concluded from experiments on rats experimentally infected with *P. equorum* that the lower the amount of vitamin A administered, the greater the survival rate of the larvae and the more rapid the development; an acute pneumonia resulted in the experimental rats and the lesions were severe and acute.

Ackert *et al*[5, 6] have found that deficiencies of either vitamin A or vitamin B result in the acquisition of a larger number of *Ascaridia galli* in chickens and also in a larger average size of the worms. McCoy[7] has demonstrated that when young rats are placed on a diet deficient in vitamin A their resistance to infection with *Trichinella spiralis* is very noticeably reduced. Foster and Cort[8] were able to increase the susceptibility of dogs and cats to nonspecific strains of hookworms by feeding diets deficient in all the vitamins and important minerals. Conversely, Shaw[9] was not able to increase the susceptibility of sheep to experimental infection with lungworms by feeding them on a vitamin A-deficient diet.

However, in only one of the vitamin A-deficient animals, Horse No. 10, was

[2]Hiraishi, T.: Experimentelle Infektion junger Schweine mit Ascariden mit Rücksicht auf die besonderen Beziehungen zur Avitamose. In Hata, S., Neuere Arbeiten ueber Parasitologie in Japan. Arch. Schiffs.-und Trop.-Hyg., xxxii (1928), pp. 519-520.

[3]Clapham, P. A.: Ascariasis and vitamin A deficiency in pigs. J. Helminthol., xii (1934), pp. 165-176.

[4]Clapham, P. A.: On the prophylactic action of vitamin A in helminthiasis. J. Helminthol., xi (1933), pp. 9-24.

[5]Ackert, J. E., McIlvaine, M. F., and Crawford, N. Z.: Resistance of chickens to parasitism affected by vitamin A. Amer. J. Hyg., xiii (1931), pp. 320-336.

[6]Ackert, J. E. and Nolf, L. O.: Resistance of chickens to parasitism affected by vitamin B. Amer. J. Hyg., xiii (1931), pp. 337-344.

[7]McCoy, O. R.: The effect of vitamin A deficiency on the resistance of rats to infection with *Trichinella spiralis.* Amer. J. Hyg., xx (1934), pp. 169-180.

[8]Foster, F. O. and Cort, W. W.: The effect of a deficient diet on the susceptibility of dogs and cats to non-specific strains of hookworms. Amer. J. Hyg., xvi (1932), pp. 582-601.

[9]Shaw, J. N.: Studies on the effect of a diet deficient in vitamin A on lung worm infestation in sheep. No. Amer. Vet., xviii (1937), pp. 25-27.

there a significant *P. equorum* infection. This may have been due to the lapse of time since removal from pastures or possibly because Horse No. 10 was more addicted to mud-eating than the other animals and consequently maintained a higher level of infection. An interesting fact is that these horses which were taken from the same pastures as the prune-fed animals, were significantly more heavily infected with *P. equorum* than the latter although they had been away from the pastures for a greater period of time; yet it must be borne in mind that they ate mud and the prune-fed horses did not.

Table III shows the results of the egg counts made for the control horses on the basis of number of eggs per gram of feces.

It is readily seen from table III that the *P. equorum* infection in the control animals was only very slightly heavier than for the prune-fed animals and very much lower than for the vitamin A-deficient horses, whereas the strongyle infection was much higher in the control animals than in the vitamin A-deficient horses but lower than in the prune-fed animals.

Although the control animals were considerably older than the two groups of test animals this does not invalidate the comparisons of strongyle infections because no evidence has been adduced that age resistance occurs against this family and Foster[10] was able to conclude from his investigations on 86 horses that there is no evidence of an age resistance of Equidae of 15 years of age or under against species of the genus Strongylus. On the other hand, however, there does appear to be a definite age resistance to *P. equorum* as shown by Baker, Salisbury and Britton.[11]

In order to determine whether or not the differences in strongyle infection between the three groups of animals were really significant the egg count data were subjected to statistical analysis by Fisher's[12] pairing method. In pairing the prune-fed horses with the control and vitamin A-deficient animals, the former were divided into two groups because of the variation in magnitude of infection in the entire group. One group (Group A) included the two most heavily infected animals but not the two most lightly infected and the other group (Group B) included

TABLE III—Eggs per gram of feces in control horses.

Date	Horse No.							
	200	201	202	203	204	205	206	207
2- 7-39	700s 0p	1950s 0p	2250s 0p	1450s 0p	0s 50p	1650s 0p	2550s 0p	1000s 0p
3- 1-39	450s 0p	850s 100p	1850s 50p	1250s 0p	150s 0p	1600s 0p	1650s 0p	650s 0p
3-15-39	900s 0p	1900s 0p	900s 0p	1200s 0p	0s 0p	700s 0p	1600s 0p	....
3-27-39	750s 0p	900s 0p	650s 50p	650s 0p	0s 0p	1550s 0p	2150s 0p	....
4-11-39	1300s 0p	1000s 50p	700s 0p	2150s 0p	50s 0p	....	1350s 0p	1350s 0p

s = Strongyle eggs.
p = *P. equorum*.

[10]Foster, A. O.: A relationship in equines between age of host and number of strongylid parasites. Amer. J. Hyg., xxv (1937), pp. 66-75.

[11]Baker, D. W., Salisbury, G. W. and Britton, J. W.: Control of equine strongylosis, part I. The effect of natural factors on the development of strongylosis in foals. Cornell Vet., xxix (1939), pp. 297-308.

[12]Fisher, R. A.: Statistical Methods for Research Workers, 4th ed., Edinburgh and London (1932), pp. 111-114.

the two most lightly infected horses but not the two most heavily infected. In order to increase the *n* values in the calculations, thereby increasing the accuracy of the analyses, each of the two egg counts for each date, rather than the average of the two counts, was employed. In the comparisons of both groups of the prune-fed horses with both the vitamin A-deficient and control animals, the differences were found to be very highly significant as also were the differences between the vitamin A-deficient horses and the controls. It can, therefore, be accurately concluded that the horses on the prune diet were more susceptible, as manifested by an increased rate of reproduction of the worms, to strongyles than the control horses kept part of the time on irrigated pastures and part of the time in the barn. This fact is emphasized by the prune-fed animals having had a slightly lower egg count before being put on the prune diet than the control horses. It must be borne in mind, however, that both of these two groups were lightly infected, but there is no reason to believe that heavier infections would have behaved differently. The infection in the vitamin A-deficient horses was very significantly lighter than that in the control animals but it must be remembered that the latter had not been on pasture for about a year, which would easily have permitted the infection to be eliminated, and although these animals did practice mud-eating the safest conclusion to draw in this instance is that but very little infection with strongyles existed and therefore no statement can be made concerning the influence of a vitamin A-deficient diet upon strongylosis.

The results obtained from the study of the horses placed on a prune diet are rather surprising inasmuch as prunes are a good source of vitamins B_1 and G and a fair source of vitamin A. Also they are adequate in minerals. The most outstanding difference between the prune and normal diets is the large amount of water and sugar present in the former. It may be that the increased fermentation which might be brought about in the alimentary canal of the host by these larger amounts of water and sugar may render the animal more susceptible to the parasite but further experimentation is necessary before a definite statement can be made. It is not known, of course, whether the prune diet had a favorable effect upon the strongyles or an unfavorable effect upon the so-called "protective antibodies," which if present would probably be of the nature of an ablastin, *i.e.*, a reproductive inhibiting antibody, since the egg count markedly increased after the initiation of the prune diet without opportunity of reinfection.

No conclusions can be drawn concerning the *P. equorum* infections because 1) not enough *P. equorum* were present in the prune-fed horses and 2) the control animals were sufficiently older than the vitamin A-deficient horses to prohibit comparison because age resistance to *P. equorum* is so marked. It is strange that the prune-fed horses were much less heavily infected with *P. equorum* than the vitamin A-deficient animals but it is well known that vitamin A deficiency greatly increases susceptibility to this family of parasitic worms and furthermore the latter group of horses indulged in mud-eating, thereby increasing their chances of becoming reinfected after the initiation of the deficient diet, although this possibility was not manifested in the case of strongyle infection.

CONCLUSIONS

1) Horses placed on a prune diet became more susceptible to strongyle infection than control animals. No data were obtained concerning the effect of this diet upon *Parascaris equorum* infection.

2) Horses kept on a vitamin A-deficient diet seemed to possess a greater susceptibility to *P. equorum* as would be expected from existing knowledge concerning the effects of vitamin A-deficiency on susceptibility to various parasites belonging to this same family. However, the vitamin A-deficient experiment was incidental and not set up in such a way as to permit definite statements concerning the effects upon strongyle and *P. equorum* infections.

A Note on Immunologic Reactions Associated with Equine Infectious Anemia*

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DURING a limited outbreak of equine infectious anemia[1] affecting a small number of horses at the laboratory farm, the absence of and need for a satisfactory diagnostic test became apparent. Experiments were accordingly initiated to determine whether under certain conditions specific immunologic reactions could be induced in actively infected or carrier horses that would be of diagnostic significance in differentiating diseased from normal animals. The procedures used are outlined with the hope that they will stimulate further study.

MATERIAL AND METHODS OF PREPARATION

The material was from two definite cases of infectious anemia. Horse No. 405 developed typical symptoms spontaneously and horse No. 302 after inoculation with infectious material from No. 405. Blood serum from both animals was stored without preservative in the cold. A part of each clot and later of specimens of spleen, liver, suprarenal, lymph node, and hyperplastic bone marrow were preserved by freezing, the remainder with 50 per cent glycerol at 4°C. Normal serum was obtained from horse No. 460 which had been under observation for seven months. Daily temperature readings and two blood counts during this period failed to reveal any abnormalities.

The method of purifying bacterial toxins developed by Wadsworth and Quigley[2] and that used by Laidlaw and Dunkin[3] for the preparation of distemper vaccine were employed to concentrate and inactivate the antigenic substances in the samples.

Method of Acetone Precipitation.[3]—Portions of blood clot, hyperplastic bone marrow, spleen, liver, and lymph node were weighed, reduced to a pulp, and mixed with sufficient 0.85 per cent sodium chloride solution to make a 5 per cent suspension. Two volumes of absolute acetone were added with constant shaking. The precipitate was recovered by filtration through paper and when dry was taken up in distilled water, chilled in an ice bath, and centrifugalized. Dialysis in cold, running tap water for three days was followed by reprecipitation with acetone. The final solution of the second precipitate was passed through Mandler filters and tested for sterility.

In the case of serum, the total globulins were first precipitated by half saturation with ammonium sulfate. The euglobulin was separated from the pseudoglobulin fraction by dialysis in a cellophane bag against running water and by isoelectric precipitation. Subsequent treatment of all serum fractions was the same. The material to be precipitated was added to nine volumes of acetone, thus keeping the concentration of the reagent always above 90 per cent. With the first sample the precipitate was removed by filtration through a Büchner funnel. Later Whatman No. 50 paper in a 60° funnel was used. The acetone was removed rapidly by washing the precipitate with petroleum ether. To facilitate the solution of the material the preliminary dialysis after treatment with ammonium sulfate was shortened to three days. Any remaining salt was precipitated

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[1]Griffin, C. A., and Brose, C. P.: Report of an outbreak of equine infectious anemia, with observations on blood changes. J.A.V.M.A. lxxxix (1936), pp. 664-670.

[2]Wadsworth, A., and Quigley, J. J.: Studies on the bacterial toxins. Concentration and purification of the toxin of *Streptococcus hemolyticus* with acetone. J. Immunol. xx (1931), pp. 459-462.

[3]Laidlaw, P. P. and Dunkin, G. W.: Studies on dog distemper-IV. The immunization of ferrets against dog distemper. J. Comp. Path. & Therap. xli (1928), pp. 1-17.

by the acetone which permitted the solution of the globulins by a second dialysis. After the addition of dry sodium chloride to give a final concentration of 0.85 per cent and centrifugalization to remove insoluble material, the product was sterilized; the first batch, S-1, by filtration through a Seitz and then a Mandler filter; all others by the addition of merthiolate to give a concentration of 1:5,000.

Three separate batches (S-1, S-2, S-6) were prepared from the total globulin fraction of the serum of horse No. 302, and two, designated S-5-I and S-5-II, from the euglobulin and pseudoglobulin fractions respectively. Control material designated as S-3 was prepared similarly from normal serum.

Treatment with Formalin.[3]—Twenty per cent suspensions of blood clot, bone marrow, spleen, and liver in 0.85 per cent sodium chloride solution were prepared, and sufficient formalin was added to give a final concentration of 0.1 per cent formaldehyde. After four days in the cold, samples were withdrawn for bacterial tests and the formaldehyde was neutralized by the addition of ammonium hydroxide.

Portions of each lot of material prepared from tissue by either method were heated at 60° C. for two hours to serve as controls.

RESULTS OF THE TESTS

Material* from the two infected animals, prepared as described, was tested by the ophthalmic or the subcutaneous method or by both in horses having or suspected of having infectious anemia and in two supposedly normal horses. Antigen S-3 prepared from the serum of a healthy horse was also tested in five apparently normal animals. The urgency of eliminating the infection as quickly as possible prevented the use of further control animals.

Material prepared by acetone precipitation from the macerated blood clot from experimental horse No. 302 failed to induce reactions when tested twice in the eye or once subcutaneously near the mucocutaneous border of the vulva of one of the suspected horses, No. 423. Following the subcutaneous injection, however, of a mixture of this material and the formalinized blood-clot extract, the horse's temperature which usually ranged from 99.2° to 100.2° F. rose to 101° F. 28 hours later.

Neither preparation of bone marrow elicited a response by any method of injection in horse No. 409, a spontaneous case. The emulsion of acetone-precipitated splenic tissue likewise induced no reaction in the eye of another spontaneous case (No. 412) or when injected subcutaneously in a horse (No. 457) infected experimentally. The acetone-treated lymph-node extract was tested only by subcutaneous injection in an experimental case (No. 465). No response was elicited. Other tissue preparations were not tested.

Twenty cubic centimeters of antigen S-1 prepared from the total globulin fraction were given subcutaneously to an experimentally infected horse (No. 387) and the temperature was followed at two-hour intervals for 48 hours. It rose from 100.4° to 101.8° F. after 16 hours and then subsided to within normal limits after 44 hours. Thirty cubic centimeters of a second batch (S-2) were injected subcutaneously into each of seven horses, four with definite clinical symptoms, three with atypical and questionable symptoms. In all of the typical and in two of the doubtful cases, temperature elevations were observed which varied from 101.4° to 105.6° F. within 42 hours. Subsequently this and a third batch (S-6) were tested repeatedly on one of the latter horses and on a clinical case, No. 399. A typical rise in temperature from 101.6° to 104.7° F., occurred in each instance. For purposes of control, horse No. 474 was also tested subcutaneously. This animal had been received in poor condition and had developed a temperature four days prior to the test but had not been suspected of suffering from infectious anemia. A temperature of 101.8° F. developed and the horse was destroyed as a precautionary

*The serum used in the majority of the tests was from the experimentally infected horse (No. 302) in which the initial reaction developed about three weeks after the inoculation. The blood used in preparing the antigen for the experiment was obtained about nine days after the subsidence of the fever reaction when the horse was bled out.

measure. Horse No. 324, which had been under daily observation for four years and was considered definitely free from the disease, was then tested. No significant rise in temperature occurred (100.4° F.). Antigen S-3 prepared similarly from the normal horse serum induced no significant reaction when 30-cc. amounts were tested on two infected and five healthy horses.

It seemed desirable to fractionate further the reacting substance of the total globulin fraction, so antigens S-5-I and S-5-II were prepared. Both were tested repeatedly in varying dosage (from 30 to 66 cc.) in a clinically typical case, No. 455, without significant results. During the intervals between injections this horse reacted to antigens S-2 (101.6° to 105.6° F.) and S-6 (102.3° F.).

DISCUSSION

The rise in temperature that occurred in all definite cases of infectious anemia and in two of the three suspected cases following subcutaneous injection of a preparation of serum from a horse ill with the disease suggests a reaction of a specific nature. This is strengthened by the fact that the same material failed to evoke a similar reaction in a horse known definitely to be free from the disease and that material prepared similarly from the serum of a healthy horse did not induce reactions in either infected or healthy animals. The temperature elevation that developed in the recently acquired horse which was in poor condition is of doubtful significance. That it indicated a latent case of the disease or was a nonspecific reaction is possible. The fever that followed the single injection of the blood-clot preparations is also of doubtful significance.

Obviously the use of only one definitely noninfected horse as a control was insufficient to establish the specificity of the test. Moreover, before the procedure described is attempted on other than an experimental basis under conditions of strict quarantine and isolation, the possibility of the virus's still being viable and of the induction of a carrier state must be investigated. The place in the fever cycle at which the serum is most likely to contain the reacting substance also requires study.

SUMMARY

In horses affected with infectious anemia elevations of temperature followed the subcutaneous injection of a preparation of globulins obtained from the serum of an infected horse but did not follow the injection of a similar material from a normal horse. The failure of the single control horse, known to be free from the disease, to react to either preparation is, however, insufficient evidence on which to establish the specificity of the test. Further investigations along these lines are suggested.

Growth Changes in Body Organs. Part II. Growth Changes in the Thyroid of the Normal Dog*

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THE VETERINARIAN and workers on endocrine organs are constantly confronted with the question as to what is the normal appearance of the thyroid gland at a given age. Much has been written in regard to the thyroid gland in relation to its histology and pathology. Most of the work has not covered a systematic study of growth changes which would be essential for the interpretation of the glandular picture at different ages. In order to remedy this apparent deficiency a series of studies has been instituted to fill in the obvious gaps in the literature. As a basis for study, dogs were selected at random as a source of material. The present manuscript covers studies on the dogs so selected. As material accumulates the effect of breed, size, sexual status, nutrition, and season of the year will be studied.

REVIEW OF LITERATURE

In 1907 Marine[1] stated that the normal thyroid gland for all mammals showed only minor histological differences, and in a later work[2] he described the microscopic unit of the thyroid as being the individual follicle, similar in all animals from fish to man. The follicles were described as enclosed spaces of variable size lined with a single layer of low cuboidal epithelium and not resting on a true basement membrane.

As early as 1889 Langedorf[3] demonstrated two cell types in the follicles of calves and dogs, the chief and the colloid cells. These two types were observed in amphibians, reptiles, birds, and mammals. The chief cells were described as being more abundant and possessing larger nuclei than the colloid cells.

The thyroid gland itself is not static histologically, for Rice,[4] working with human material, has definitely illustrated the variation of the microscopic structure. These variations depended somewhat upon age and locality, according to Rice, and as a person passed through life the thyroid increased in weight reaching its maximum during early adult life after which it again decreased gradually to the end of the life span. The follicles (acini) increased or decreased in size as the weight of the gland changed, and the acinar epithelium varied from flat to cuboidal, but columnar cells were not thought to be normal.

Cooper,[5] who correlated the structure of the human gland with various periods of life, concluded that during early intrauterine life the vesicles were developed from solid epithelial masses, but in later fetal life the epithelial cells became active after which there was a resting phase. The epithelium was found to be very high during childhood but in the adult the follicles were very large and distended with colloid. As age increased follicles became smaller and decreased in numbers.

*Department of Veterinary Anatomy, Veterinary Division, Iowa State College. Part I appeared in Iowa Acad. Sci., xlvi (1939), pp. 439-441. This project received material support from the Research Council of the Graduate College of Iowa State College.

[1]Marine, D.: On the occurrence and physiological nature of glandular hyperplasia of the thyroid (dog and sheep) together with remarks on important clinical problems. Johns Hopkins Hosp. Bull., xviii (1932), pp. 359-364.

[2]Ibid: The thyroid, parathyroid, and thymus, Cowdry's Special Cytology (1932), pp. 799-820.

[3]Langendorf, O.: Beiträg zur Kenntniss der Schilddrüse. Arch. f. Anat. u. Physiol., Suppl.-Bd. (1889), pp. 219-242.

[4]Rice, C. O.: Histologic structure of the normal thyroid gland. Arch. of Surg., xlix (1938), pp. 96-101.

[5]Cooper, E. R. A.: The Histology of the More Important Human Endocrine Organs. Oxford Med. Publ. (1925).

Abbott and Prendergast[6] studied the histological variations in animal thyroids in western Canada. Their material included thyroids of young calves, yearling cows, old range cows, mink, foxes, sheep, swine, buffalo, and horses. In the young animals of these different species, active hyperplastic glands were the rule and were not considered as being caused by any pathological condition. These workers found as age advanced that the acini tended to become larger varying in size and not in number, colloid increased in amount, and in the older animals there was a picture which resembled that of the normal gland. The glands in very old animals had greater amounts of colloid, the follicular cells tended to become flattened, interacinar fibrous tissue increased, and the generalized picture was of a gland "past its prime, sluggish, and gradually declining to decay and death." Contrary to what Cooper found in her above-mentioned work, these researchers, Abbott and Prendergast, contended that free cells did not occur normally in the colloid. They found that there was always a single layer of follicular epithelium. Vacuolation occurred at any age and was present before birth, follicles in the animals of the old-age group were found to be distended with colloid and not smaller than the earlier ages. Abbott and Prendergast confirmed the observations of Cooper, that histological thyroid variations may take place normally during the life span of animals.

THE THYROID OF THE DOG

The thyroid gland in the dog consists of two lobes located on the lateral surface of the trachea near the larynx. In the larger dogs an isthmus consisting of a wide glandular band connects the two lateral lobes, but in smaller dogs the isthmus is usually absent. Each lobe of the thyroid usually has two parathyroids related to it, the posterior one is often imbedded in the thyroid parenchyma.

Halsted[7] described the normal canine thyroid in conjunction with an experimental work. He said, "The follicles as they appear in section vary in size more than in shape. They incline to roundness in shape and are lined by a single layer of cubical, sometimes almost flat epithelium and are filled with a homogeneous substance called colloid."

In the previously cited work of Marine[1] it is stated:

> In the dog the normal gland does not exceed 0.300 gm. per kilogram of body weight. The gland capsule is thin. The vessels are small. Microscopically the acini are found, slightly variable in size with regular walls. There are no intra-acinar twigs or projections. The stroma is scanty and supports very small blood and lymph vessels. The epithelial cells lining the acini are regular, low cuboidal, with small darkly staining nuclei, and are arranged in a single layer. The stainable colloid is dense, homogeneous, and stains uniformly. It sharply abuts the epithelial cells.

These early publications have included the most important factors in the descriptions concerning thyroid structure, and though not specific they give a general picture of the canine thyroid.

MATERIAL

The material here includes a series of 70 dogs which were selected at random. The group consists of purebred dogs, crosses, and mongrels, all of which were apparently free from disease. The body weight, breed, age, sex, and the thyroid gland weight were compiled for each individual specimen.

As this study is outlined only to determine the effect of age upon the growth changes of the thyroid gland, the factors which might also effect this growth change such as size, sexual status, season of the year, nutrition and iodine content of the thyroid gland have been disregarded.

In table I the average weight of the thyroid glands per kilogram of body weight for the various age groups of animals used

[6] Abbott, C., and Prendergast, J.: Histological variations in animal thyroids in western Canada. The Canad. Med. Assn. J., xxxi (1934), pp. 465-473.

[7] Halsted, Wm. S.: An experimental study of the thyroid gland of dogs with especial consideration of hypertrophy of this gland. Johns Hopkins Hosp. Rpts., i (1896), pp. 373-408.

TABLE I—Average weight of the thyroid glands per kilogram of body weight for the various age groups of animals used.

AGE GROUP	GLAND AVERAGE PER KILOGRAM BODY WEIGHT	AGE GROUP	GLAND AVERAGE PER KILOGRAM BODY WEIGHT
3 days	0.293	28 weeks	0.184
4 days	0.260	36 weeks	0.191
6 days	0.330	1 year	0.064
3 weeks	0.188	1 year, 54 days	0.097
4 weeks	0.098	3 years	0.073
5 weeks	0.460	4 years	0.098
6 weeks	0.342	6 years	0.097
8 weeks	0.136	11 years	0.047
10 weeks	0.182	12 years	0.081
12 weeks	0.085	13 years	0.080
14 weeks	0.240	15 years	0.092
16 weeks	0.134		

has been summarized. In the case of young animals not weighing 1 kilogram the ratio was computed by

$$\left(\frac{1\text{ kg.}}{\text{body wt. in kg.}}\right) \times \text{(the gland weight (Gm.)}$$

and where the body weight exceeded 1 kilogram, the formula,

$$\left(\frac{\text{gland wt. (Gm.)}}{\text{body wt. (kg.)}}\right)$$

was used. Since there is a wide variation in weight of dogs at the same age because of breed and other factors, then when the gland weight per kilogram of body weight is calculated one specimen can be compared with another, and the age groups averaged.

It has been mentioned in the discussion of the works of Cooper, Rice, and Abbott and Prendergast that the histological structure of the thyroid changes with age, and such seems to be true of the dog when both thyroid histology and weight are studied. The large glands per kilogram of body weight occur in dogs from birth to 6 weeks of age. This is followed by an interval of variation. There does, however, appear to be a slight decrease in gland weight per kilogram of body weight in the older dogs.

HISTOLOGY

In this discussion of the thyroid gland at various ages, the group at one given age will be described, but special note of any individual differences will be included.

The 25-Day-Old Fetus.—The fetal thyroid presents a specific developmental picture (fig. 1). Those follicles that have formed contain colloid of a uniform texture and in this substance there are very small vacuoles. The follicle diameters range from 15-40μ (table II). The gland is extremely vascular, and the nuclei of the follicular epithelium are large with only relatively small amounts of cytoplasm surrounding them. The epithelium is 8.83μ in height (table II) even greater than in any group after birth up to the 12th week. There are many areas of undifferentiated cells where the follicles have not formed. Mitotic figures are numerous.

The Full-Term Fetus.—At full term (fig. 3) the follicles are very numerous; though they vary in size from 15-50μ, most are of a diameter less than 50μ and average, in general, 31μ. Colloid is present in all follicles, and it may be stated here that no follicles examined, with the exception of those of the 25-day-old-fetus, were ever completely without colloid. Two cell types are present in the follicular epithelium from full term to the oldest specimen. The follicular epithelium average 6.3μ in height. There are fewer mitotic figures than in the 25-day-old-fetus. Vacuolation, as in all other groups, seems here to be constant.

The 6-Day Group.—The follicles are of a

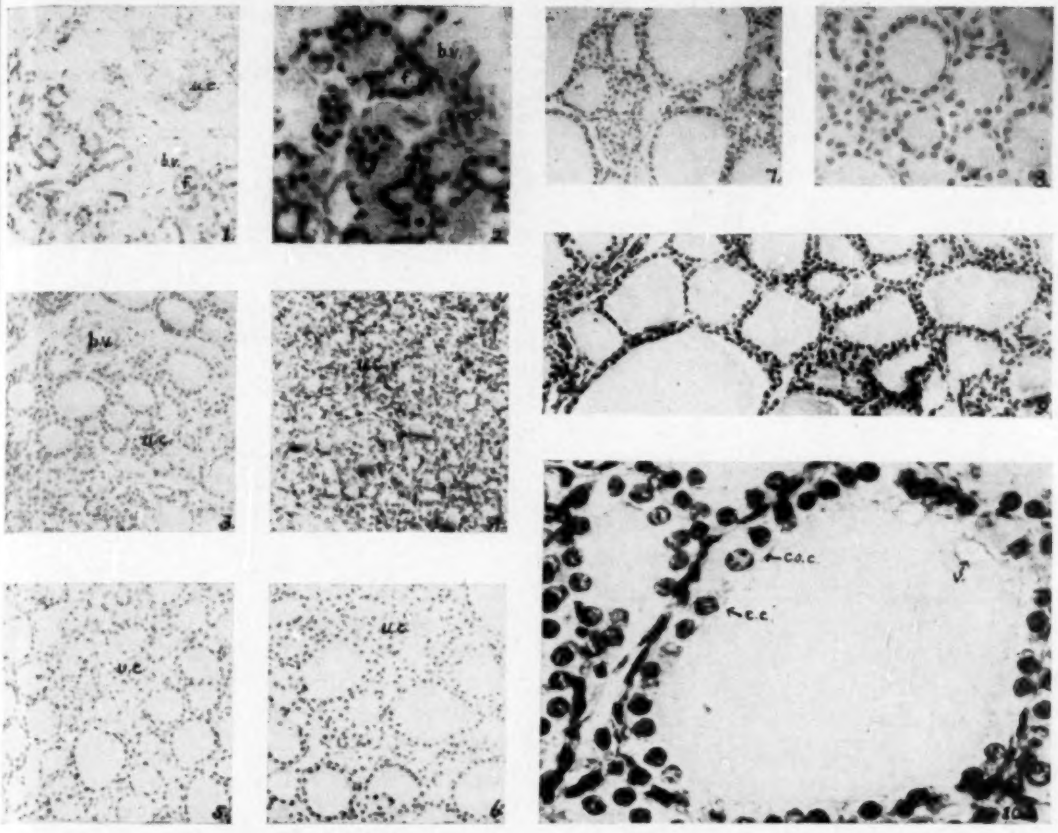

Fig. 1. Section of the thyroid gland of a 25-day canine fetus (x 188). H. and E. Fig. 2. Section of the thyroid of a 25-day-old dog ((x 376). Undifferentiated cells, few follicles, cuboidal follicular epithelium with large nuclei and marked vascularity are shown. H. and E. Fig. 3. Section of the thyroid gland of a full-term canine fetus (x 188). It shows large numbers of follicles, low cuboidal follicular epithelium and small areas of undifferentiated cells. H. and E. Fig. 4. Section of the thyroid gland of a 6-day-old dog (x 188). Section shows more constant size of follicles, low cuboidal follicular epithelium and small areas of undifferentiated cells. H. and E. Fig. 5. Section of the thyroid gland of a 4-week-old dog (x 188). Section shows increase in follicular size and the absence of very small follicles. Areas of undifferentiated cells are still present. H. and E. Fig. 6. Section of the thyroid gland of a 5-week-old dog (x 188). This section compares closely with figure 5. H. and E. Fig. 7. Section of the thyroid gland of an 8-week-old dog (x 188). This section shows the increase in follicular size as compared with the preceding age groups. H. and E. Fig. 8. Section of the thyroid gland of a 10-week-old dog (x 188). This section shows the high cuboidal follicular epithelium characteristic of this age group. H. and E. Fig. 9. Section of the thyroid gland of a 16-week-old dog (x 188). H. and E. Fig. 10. Section of the thyroid gland of a 16-week-old dog (x 752). This section shows many large follicles, high columnar follicular epithelium, vacuolation, colloid and chief cells. H. and E.

ABBREVIATIONS

u. c—undifferentiated cells
b. v—blood vessel
co. c—colloid cell
c. c.—chief cell
v—vacuole
f—follicle

TABLE II—Average follicular diameters and epithelial heights.

AGE	AVERAGE FOLLICLE DIAMETER (μ)	AVERAGE HEIGHT OF EPITHELIUM (μ)	DIAMETER FOLLICLE VARIATION (μ)	VARIATION OF EPITHELIAL HEIGHT (μ)
25-day fetus	30.83	8.83	15–60	5.8–11.0
Full term	31.00	6.30	15–70	5– 7.0
6 days	32.70	7.35	20–50	6–10.0
4 weeks	40.00	7.92	25–85	6–11.2
5 weeks	59.00	10.92	30–80	8–14.4
6 weeks	64.00	7.90	30–120	4.5–12.4
8 weeks	67.18	7.94	27–138	5–13.0
10 weeks	68.30	7.38	30–140	6–10.8
12 weeks	75.30	9.56	40–120	7–12.6
16 weeks	76.10	12.71	30–160	8–20.0
28 weeks	75.30	10.78	30–150	7–18.0
1 year	70.00	11.00	25–155	5–18.0
6 years	72.00	10.91	30–150	5.5–15.0
11 years	70.10	10.00	10–130	5–15.0
13 years	110.00	6.30	30–220	5–15.0
15 years	99.10	7.53	30–200	6– 9.0

more constant size averaging 32.70μ at this age, and although the largest follicles of the previous group are larger than those contained in this group there is not a wide variation. The height of the epithelium is 7.35μ and there is some undifferentiated tissue between the follicles.

The 4-, 5-, 6- and 8-Week Groups.—In these four groups regardless of the variability recorded in connection with glandular weights, there is no great histological difference. There is an increase in follicular size as age increases, there being more large follicles measuring 110μ or better at the 8-week age than at the three preceding ages, and, too, there are less numerous small follicles. The range and average figures for the diameters of the follicles for the four groups are recorded in table II. The large follicles have lower epithelium than the smaller, and this is a trend true in each of these four ages, a factor especially evident through all the other age groups. From table II it can be noted that there is no constancy of epithelial height but no cells within these follicles are higher than a cuboidal type. The period in which the increase in size of the follicles is most marked is from birth to 8 weeks, the greatest increase occurring at 6 weeks.

The 10-Week Group.—Results recorded in table II indicate that larger follicles appear at 10 weeks of age than at 8, and as figure 8 illustrates, the epithelium is high and cuboidal. No great degree of vacuolation occurs, but some does appear at the usual peripheral location. The colloid is dense and strongly eosinophilic.

The 12-Week Group.—The follicles are markedly enlarged and the epithelium is increased in height. However, the appearance of large cells apparently located between the follicles, but which Halsted[7] and others class as parts of the follicular apparatus, may be taken as an outstanding variation from the previously described glands.

The 16-Week Group.—The follicles in this group have become as large as 160μ in diameter and many of this width are present, but those smaller than 160μ preponderate. The epithelium is higher than at any previous period, but that tissue in the large follicles may be as low as 8μ. The range and average figures for follicle diameters and epithelial height are given in table II. The thyroid of one breed in this group, the Beagle Hound, does not closely resemble the other breeds in that it contains "interfollicular" masses similar to those noted in the 12-week group. These so-called interfollicular cell masses are probably parts of other follicles or areas where young follicles may develop. The

cell types in the follicular epithelium are easily demonstrated (fig. 10). The colloid cells follow the classical description of Langendorf, the chief cells being smaller and more densely nucleated than the larger colloid cells. Venzke[8] has described the 16-week-old canine thyroid as a gland typical of the species, which would, for comparison with other species, constitute an arbitrary norm.

The Older Animals.—Although the thyroids of a 9-month-old Fox Terrier and the 6-year-old group are (table I) heavier per kilogram of body weight than the remainder of the material in this older group, which ranges in age from 7 months to 15 years, when compared with the 16-week gland, no great difference in histological structure can be seen. This is not true of all the members of this series, however, because a 2-year-old Fox Terrier female, in heat, had very large strands of epithelial tissue seemingly located between the follicles, but this tissue may be either parts of the follicles or areas where young follicles may develop. Nonidez[9] reports in his work on the canine thyroid that cells may arise from the follicular epithelium and migrate to a point between the follicles. Another variation in the older group was found in the gland of a 4-year-old English Bull Terrier. Here the epithelium was of a columnar type and showed some infolding. The other specimens resembled the 16-week group but had a wider range of follicular diameter, since more very small vesicles were present. Table II gives representative measurements taken from typical members of the group. In the gland of the 11-year-old dog areas of blue staining substances in the eosinophilic colloid are occasionally noted.

Discussion and Summary

The larger thyroids which occur in the younger dogs, estimated in terms of grams per kilogram of body weight, may be explained by the growth processes, thyroid requirements in these young animals, or insufficient iodine supply in the mother's diet during pregnancy. From the data here computed one might assume that these glands were in an early phase of great activity, but since weight is only regarded as one factor in estimating thyroid activity, epithelial height should not be overlooked. These measurements (table II) show great variability, but if the work of Abbott and Prendergast is recalled, these deviations may be expected. However, the glands in the old animals are lighter in weight comparatively on the average, where the growth process has ceased. The follicular diameter in small animals increases in a uniform manner to the 16th week. After this age some follicles are larger than at 16 weeks, but many are of the size of this latter group.

Individual differences do occur within a group when several breeds are used, and because of those diversities in glandular structure, the genetic make up of the dog is thought to play a rôle in the histology of the thyroids here studied.

The much discussed "interfollicular" tissue was studied by the use of silver impregnation for reticular tissue in an attempt to bring out the limits of follicular and interstitial boundaries. The reticulum forms a boundary for most of the follicles, but seemingly is not constant, for reticular fibers can be followed enclosing groups of follicles which have no reticular separation. Too, "interstitial tissue" composed of cells not resembling the chief cells, but instead, the colloid cells are also encircled, and oftentimes within these areas minute amounts of colloid occur, and it is because of this observation the writers feel these so variously named cells located between the follicles are either portions of the developed follicle cut on a tangential plane, or areas where new follicles may be formed.

There are two cell types present in the canine thyroid, a fact long known, but the function of these is debatable. Perhaps the cells exhibiting these structural differences

[8] Venzke, W. G.: The histology of the thyroid gland of dogs sixteen weeks of age. Iowa Acad. Sci., xlvi (1939), pp. 439-441.

[9] Nonidez, J. F.: Origin of the parafollicular cell, a second epithelial component of the thyroid gland of the dog. Amer. J. Anat., li (1932), pp. 479-505.

are of one and the same type showing the effects of age, intensity of functional activity, and special environmental conditions. Bensley[10] believes there are two separate functions which these cells perform, but Marine[2] maintains that just different phases in the secretory process are presented.

Eggert[11] stated that single cells may occur in the reticular fibers of the thyroid which have a phagocytic function. It has also been noted here that single cells occur along the reticular framework of the gland. These are quite large granular cells; they resemble the Von Kupffer cells of the liver.

In the older animals the glands are usually found to resemble the 16-week age, but before this time great variations occur; so the reason why the 16-week gland is chosen as typical is that the older thyroids are usually similar; hence the histological findings at 16 weeks would be present over a greater part of the life span of the dog.

[10]Bensley, R. R.: The normal mode of secretion in the thyroid gland. Amer. J. Anat., xviii (1916), pp. 37-53.

[11]Eggert, B.: Morphologie und Histophysiologie der normalen Schilddrüse. Johann Ambrosius Barth-Verlag-Leipzig.

CONCLUSIONS

On the basis of the evidence presented the following tentative conclusions may be drawn concerning the growth changes of the thyroid of the dog:

1) There are wide variations in thyroid-gland weight per kilogram of body weight.

2) The follicles increase in diameter in a constant manner up to 16 weeks of age.

3) The epithelium of the follicles is extremely variable. Even when the follicles are increasing in diameter in a constant progression to the 16-week period, the epithelial cells varied; the thyroid is not a static unit.

4) Histologically there are two types of follicular cells, which are first noted in the full-term fetus. Functionally these may be of one and the same type of cell.

5) Colloid appears in the 25-day-old fetus together with small vacuoles.

6) The follicle may or may not be completely surrounded by reticular fibers; there is no true basement membrane.

A Review of the Life History of Spirocerca Lupi (=S. Sanguinolenta), the Esophageal Worm of the Dog*

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Recently Brown and Austin[1] reported that they have confirmed the findings of Mundhenk and Greene[2] and that there is a danger of confusing the microfilaria of *Dirofilaria immitis* with immature stages of *Spirocerca sanguinolenta*†. Since Khaw and Cheu[3] were able to clear the blood stream of microfilariae in cases of coincident infection with *D. immitis* and *S. lupi*, the reports of the first mentioned writers[1, 2] are open to question. On reviewing the literature dealing with *S. lupi*, it would appear that the experimental data available[4, 5, 6, 7, 8, 9, 10, 11, 12] practically preclude the possibility that this worm could produce microfilariae.

Since Mundhenk and Greene[2] have reported the production of microfilariae by *S. lupi*, and this has been accepted as a fact by at least two authors,[1, 13] it seems advisable to review briefly the pertinent data on the life history of this parasite.

Life History

Since the structure and organization of *S. lupi* is typical of the Spiruroidea in general, it is to be expected that the life history of this form would be typical of this group as a whole. In the life history of this group the embryonated eggs are eliminated in the feces but in most cases do not hatch until ingested by a suitable arthropod, frequently a coprophagus insect. After a period of development in the arthropod intermediate host, the larval worms encyst in this host and are then infective to a suitable definite host. Infection occurs usually when the definitive host ingests the invertebrate intermediate host.

The adult of *S. lupi* is found usually within tumor-like nodules in the esophagus.[5, 6, 7, 9, 13] In most cases the cavity of the nodules communicates with the lumen of the esophagus through a small, smooth, rounded aperture. A milky purulent fluid, containing the eggs of the parasite in all stages of development, exudes from this aperture; the eggs are also found in the exudate within the tumor[9]. The eggs in the feces of the infected dog host measure 22 to 27μ by 8 to 12μ and are usually fully embryonated (fig. 1a) or nearly so.[6] The larvae in the eggs from the feces may be expressed by exerting slight pressure upon an embryonated egg mounted under a coverglass.[6] The larvae (fig. 1b) show a well-developed esophagus and intestine and certain peculiarities of the head and tail, which are never seen in the more primitive, undifferentiated microfilariae of *D. immitis* (fig. 1c).

The eggs from the feces of the final host will not hatch in water, feces or soil[6] but will hatch when eaten by a coprophagus beetle.* In the arthropod intermediate host, the larvae as already stated, develop to a stage which is infective to the final host.[5, 11] The infected beetles are commonly eaten by vertebrate hosts other than the dog. While the larvae are incapable of developing to the adult stage in these unusual hosts, they do not perish but become encapsulated in the mesentery or other tissues and remain infective for the dog or other suitable definitive host.[4, 5, 8, 9, 11] These encysted forms have been reported from a number of vertebrate hosts[4, 5, 8, 9, 11, 12]

*Food and Drug Administration, U. S. Department of Agriculture. The aforementioned administration, except for its functions associated with the Insecticide Act of 1910 and the Naval Stores Act, was transferred to the Federal Security Agency on June 30, 1940.

†The correct name of the esophageal worm of dogs is *Spirocerca lupi* (Rudolphi, 1890). See Chitwood, J. Parasit., xx (1933), p. 69.

*Faust[5] and Ono[10] have disproven Grassi's[14] earlier report that cockroaches serve as intermediate hosts for *S. lupi*.

many of which are commonly eaten by dogs.

Upon being swallowed by the dog, either with a beetle or with a vertebrate (second intermediate or transfer host), the infective larvae of *S. lupi* are freed in the stomach by gastric digestion and migrate to their final location.[4, 5, 8, 9, 10, 11] Faust[4, 5] reported that the route of migration was by way of the portal circulation and through the capillaries of the liver and lungs. Hu and Hoeppli[8], however, were able to show

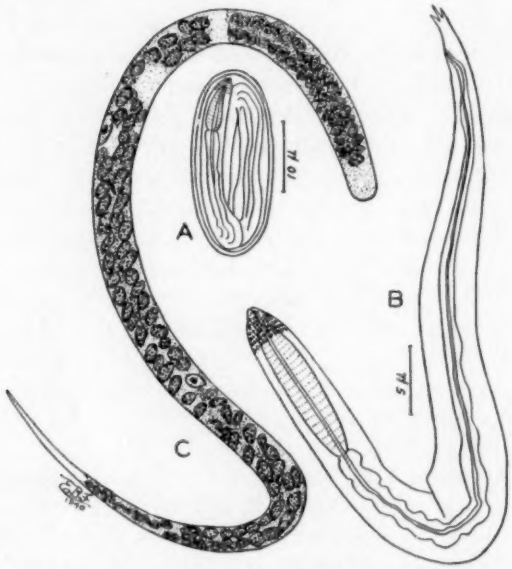

Fig. 1. A—egg of S. lupi containing larva (after Faust, 1929), B—larva of S. lupi from the egg shell (after Faust, 1929), C—microfilaria of Dirofilaria immitis from blood of dog (x 450).

that the larvae of *S. lupi* did not migrate through the capillaries of these organs but, when fed experimentally to dogs, became free in the stomach cavity and penetrated directly into the stomach wall. After the larvae reach the serosa, many of them migrate in the walls of the coronary (gastric), gastroepiploic and celiac arteries toward the upper abdominal and lower thoracic, and finally the upper thoracic portion of the aorta. This migration is chiefly in the walls of the vessels mentioned. From the wall of the upper portion of the thoracic aorta the parasites migrate toward the wall of the lower portion of the esophagus by way of the connective tissue which lies between these two organs; they develop to maturity in the walls of the esophagus producing the tumor-like alterations which have already been described.

DISCUSSION

Mundhenk and Greene[2, 15] determined by postmortem examination that a large percentage of dogs showing microfilariae in the blood stream harbored adults of *S. lupi*, but no adult filariid worms. This suggested to them that the microfilariae that they had observed might be immature stages of *S. lupi*. Unfortunately, the suggestion of Mundhenk and Greene[2, 15] is based on no data other than the coincident occurrence of adult specimens of *S. lupi* in most, but not all, cases in which microfilariae were present in the blood. The present writer does not attempt to explain the origin of these microfilariae, but on the basis of available information it appears certain that they are not immature stages of *S. lupi*.

Since the life history of *S. lupi*, seems to be typical of spirurids in general,[4, 5, 6, 7, 8, 9, 10, 11, 12] it is unlikely that the life history of this species should include a stage which circulates in the blood stream. In all cases in which a nematode occurs commonly in the blood stream, a blood-sucking arthropod is required as an intermediate host. Therefore, it would appear that the microfilaria of Mundhenk and Greene[2, 15] is incapable of further development unless it is ingested by a blood-sucking arthropod. This type of development is typical of the filariid group and is totally different from that determined experimentally for *S. lupi*.[4, 5, 6, 7, 8, 9, 10, 11, 12] Furthermore, it is improbable that one nematode of a well-known group would exhibit two such distinctly different modes of development. Since *S. lupi* is normally a parasite of the wall of the alimentary tract, the eggs reach the exterior by way of the feces and are known to hatch only when ingested by coprophagus beetles;[6] the infective larvae of *S. lupi* migrating in the walls of the arteries could not be confused with microfilariae because of striking differences in size and structure. Moreover, the larvae of *S. lupi* are not

capable of circulating through the capillary system[8].

The complete life history of *S. lupi*, as stated by Faust,[5] includes only two hosts, the insect (primary larval) and the dog (definitive), although a wide variety of vertebrates which are either insectivorous or ingest insects accidentally may become intercalated in the life cycle as reservoir intermediate hosts.

SUMMARY

The adult esophageal worm, *Spirocerca lupi* (=*S. sanguinolenta*), is a spirurid parasite of the wall of the digestive tract of dogs and does not produce microfilariae as was suggested by Mundhenk and Greene.[2, 15]

A review of the literature dealing with the life history of *S. lupi* is presented.

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Identification of Salmonella Pullorum Colonies with Immune Serum by Means of a Macroscopic Plate Test*

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DURING the school year of 1925-1926, one of us (H. J. S.) while spending several months at the Royal Hungarian Veterinary College, had occasion to learn the use of a quick agglutination test in the identification of *Salmonella choleraesuis*. Single colonies growing on Conradi-Drigalski agar plates and appearing to be like those of *S. choleraesuis* were picked and stirred into a drop of rabbit serum highly immune to this microörganism. This was done on a glass slide. Immediate and distinct agglutination was obtained with colonies of *S. choleraesuis* but not with any other organism encountered in swine. Twiehaus[1] suggests taking several loopfuls from the growth on an agar plate and mixing them with several loopfuls of antipullorum serum for the purpose of quickly identifying *Salmonella pullorum*. If he means taking single colonies and mixing them in separate drops of serum, the method seems logical. However, it would not seem wise to mix several colonies in one drop of serum because of the possibility of making a mixture of more than one species, something that would quite surely lessen the accuracy of this procedure.

The purpose of this work was to determine whether or not a rapid agglutination (plate) test would give satisfactory identification of the pullorum organism. If such a test proves to be accurate, about 48 hours can be cut from the time necessary for making fermentation tests. Another point is that less equipment is needed.

PROCEDURE

An attempt was made to hyperimmunize hens to *Escherichia coli, Shigella gallinarum, Salmonella aertrycke* and *S. pullorum*. Two strains each of *E. coli, Sh. gallinarum* and *S. pullorum* were used and one culture of *S. aertrycke*. The vaccines were made from stock cultures of low virulence except that the second cultures of *Sh. gallinarum* and *S. pullorum* appeared to be quite virulent. The coli cultures did not produce any appreciable agglutination titre and the *S. aertrycke* titres were only 1:200 and transient. Birds immunized with *Sh. gallinarum* either died before developing a detectable amount of antibodies or developed only low titres. In the case of pullorum vaccines, sufficiently high titres to warrant considering the serums as hyperimmune were obtained in two hens. High-titred antigallinarum serum was obtained from some birds which had been used in another experiment.

Tube and plate tests were made with the homologous organisms and a series of 20 other Salmonella cultures which had been isolated from chickens. For the plate tests, the antigen consisted of growth picked up with a loop directly from the colony or streak. This was mixed with a drop of the diluted antiserum on a glass plate or slide.

The agglutinin-absorption technic was employed for the purpose of eliminating cross reactions between different strains. It was found that for practical purposes these cross-reactions could be eliminated just as efficiently by the proper dilution of the immune sera.

RESULTS

S. pullorum cultures could be differentiated from all of the organisms used except *Sh. gallinarum*. *S. pullorum* and *Sh. gallinarum* being antigenically identical, this was anticipated.

S. pullorum immune serum diluted so

*From the Department of Bacteriology, Michigan State College; journal article No. 456(n.s.) of the Michigan Agricultural Experiment Station.

[1]Twiehaus, M. J.: The diagnosis of poultry diseases. Vet. Med., xxxiv (1939), p. 698.

that the titre is about 1:50, will agglutinate pullorum and gallinarum cultures regularly (plate test) and will not agglutinate any of the other organisms used. The same is true of *Sh. gallinarum* antiserum.

The high-titre antiserums used, when employed undiluted, will sometimes give cross reactions with other organisms of the group. The reaction developed in less than a minute in most cases. If a much longer time was required, the test could not be read due to the rapid drying of the small amount of antiserum used. A titer of at least 1:500 for *S. pullorum* should be obtained in the test antiserum to permit diluting it ten times or more. Such dilutions give more distinct reactions and are more convenient.

S. aertrycke antiserum showed inconsistent results. Cultures of this type were, therefore, not identified with any degree of certainty.

CONCLUSIONS

1. The macroscopic plate test will differentiate *Salmonella pullorum* colonies from those of Salmonella organisms other than *Shigella gallinarum*. The fact that these two organisms give identical reactions with this test is no disadvantage from a practical standpoint. Such cross reactions occur in the accepted agglutination tests now employed in the control of pullorum disease and some investigators consider *Sh. gallinarum* merely a variant of *S. pullorum* thus making pullorum disease and fowl typhoid one problem.

2. The practical importance of shortening the time required for diagnostic procedures is too obvious to warrant further comment.

3. If there are no colonies of sufficient size to make this procedure possible, agar slant cultures must be made from the available colonies, and the antigen can then be taken from them after 15 to 24 hours. By so doing, one can still save enough time and materials to make this method of practical value.

A Nonspecific Lesion of the Esophagus and Oral Cavity of the Chicken*

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A LESION of the upper esophagus and oral cavity was observed in the college poultry flocks at the same time a similar type of lesion was reported to occur in farm flocks in this area. The question immediately arose as to the relationship of this condition to nutrition, especially since a deficiency of vitamin A has been reported to result in a condition which, upon gross examination, might be said to resemble the lesions under consideration here.

Seifried,[1, 2] studying the histopathology of the lesions produced by avitaminosis A, differentiated between the lesions produced in the upper alimentary and respiratory tracts and those of laryngotracheitis, bronchitis, contagious coryza and fowl pox. The essential microscopic lesion was described as a degeneration of the epithelial cells of the esophagus and mucous glands with a marked metaplasia resulting in an abnormal amount and distribution of keratonized epithelium. These changes also have been noted by Beach[3] in the chicken and Wolbach and Howe[4] in the rat.

Feldman[5] reported a case of metaplastic changes in the esophagus due to a previous avitaminosis. It was felt that a histopathological study should be made to determine whether or not the condition reported here was the same as that resulting from a deficiency of vitamin A.

EXPERIMENTAL PROCEDURE AND RESULTS

The materials used in this study were from the following flocks of the department of poultry husbandry: 1) laying hens both on the floor and in batteries, receiving a ration of natural feedstuffs adequate in all known essential nutrients; 2) laying hens receiving a vitamin A-deficient ration; and 3) laying hens receiving a riboflavin-deficient ration. The composition of the three rations employed is given in table I.

The vitamin A- and riboflavin-deficient rations were fed on the all-mash basis while the complete ration was fed with equal parts of scratch grain, the grain mixture consisting of 50 per cent yellow corn, 25 per cent wheat, and 25 per cent oats. The hens on the vitamin A-deficient ration were irradiated daily to prevent vitamin D deficiency, since the ration contained no cod liver oil. Examination of the esophagus in the living hens yielded the data recorded in table II.

It is apparent from the examination of these 475 hens that neither nutrition, age, breed, nor system of management influenced the incidence of the lesions to an appreciable extent. The lesions also were observed to occur in cockerels to approximately the same extent that they occurred in hens. No appreciable effect was observed on the egg production of these individuals.

GROSS PATHOLOGY

The lesions are grayish white, mucopurulent areas adherent to the mucous membrane of the esophagus, ranging in size from 2 to 10 mm. in length by 2 to 4 mm. in width (figs. 1 and 2). For the most part, the lesions began on the dorsal posterior section of the oral cavity, extending posteriorly into the upper portion of the esophagus. Lesions are not observed on the mucous membrane of the upper esophagus for a distance of more than 3 cm. from

*Paper No. 909, journal series, Pennsylvania Agricultural Experiment Station.

[1]Seifried, O.: J. Exp. Med. lii (1930), p. 533.

[2]Ibid: p. 519.

[3]Beach, J. R.: California Agr. Exp. Sta. Rep. (1920-21), p. 140.

[4]Wolbach, S. B., and Howe, P. R.: J. Exp. Med. xlii (1925), p. 753.

[5]Feldman, W. H.: J.A.V.M.A. xciii (1938), p. 260.

TABLE I—Compositions of the rations.

	Complete Ration Per Cent	Vitamin A Deficient Per Cent	Riboflavin Deficient Per Cent
Ground yellow corn	31.5		48.5
Ground white corn		47.5	
Wheat bran	15.0	10.0	10.0
Wheat middlings	15.0	10.0	10.0
Ground heavy oats	10.0	15.0	15.0
Meat scraps	7.5	15.0	
Fish meal	5.0		
Casein			9.0
Dry skim milk	5.0		
Alfalfa meal	7.5		
Cod liver oil	2.0		1.0
Calcium carbonate	1.0	2.0	2.0
Steamed bone meal			4.0
Sodium chloride	0.5	0.5	0.5

the pharynx. In 25 per cent of the cases the lesions appear on the mucous membrane of the oral cavity just posterior to the tongue.

Removal of the adherent mucopurulent exudate during swabbing for bacteriological examination results in slight bleeding. One of the most interesting features of the condition is its short duration. The entire lesion is observed to heal completely in five to ten days and from all gross evidence the mucous membrane of the esophagus appears normal. Table II indicates the incidence of the condition from the standpoint of gross examination. Periodic examination from October to March revealed that the lesions may persist for a period of from seven to ten days and may recur within a few days or not until after several months. In severe cases the lesions are very abundant, sometimes involving practically all of the posterior part of the oral cavity and upper esophagus. In only two cases were any lesions noted laterally to the tongue or on the palate. No marked general effect was observed in these chickens. The pustular-like lesion reportedly associated with a low-vitamin A intake in the adult bird was not observed despite the fact that one group of hens was maintained on the vitamin A-deficient ration for a period of five months. That the lesions did not develop in these hens appears of sufficient importance to justify the continuance of the study.

Histopathology

The lesions recorded in this study were confined principally to the epithelium but in a few cases the outer portion of the lamina propria was involved. In addition there was an increase in the number of lymphocytes in the numerous lymphatic nodules around the mucous glands.

The primary cellular reaction was a migration of polymorphonuclear leucocytes through the stratum germinativum to the surface layers of epithelium (fig. 3). The size of the lesions varied considerably but the tissue reaction was essentially the same.

TABLE II—Incidence.

Hens Examined		Ration	Age Laying Year	Breed	Confinement System
Total Number	Affected Per Cent				
142	53	Riboflavin deficient	1st	SCWL	Floor
46	35	Complete	2nd	SCWL	Floor
36	75	Complete	1st	BPR	Floor
94	67	Complete	2nd	BPR	Floor
145	43	Complete	1st	SCWL	Battery
12	32	Vitamin A deficient	1st	SCWL	Battery

except that the larger lesions extended deeper into the epithelial layer (fig. 4). The predominating cell type in the lesions of short duration was the polymorphonuclear leucocytes. In the cases of longer duration more lymphocytes and macrophages were found. The mucous glands were not primarily affected in any of the cases. In some instances the epithelium was necrosed and entirely destroyed around the opening of the excretory duct of the mucous glands, yet with no manifestations of changes in the gland other than an increase in the quantity of mucus. Upon serial section of the same glands they did not show any extensive injury. No metaplasia of the secreting epithelium of the mucous glands was observed. In some of the cases on the vitamin A-deficient diet a slight thickening of the stratified squamous epithelium was observed, but there was no indication of the characteristic changes which have been reported in advanced avitaminosis A.

Bacteriology

Fifteen cases of the total were cultured on blood agar and brilliant green agar. Upon the bases of morphology and the carbohydrate reactions, most of the organisms

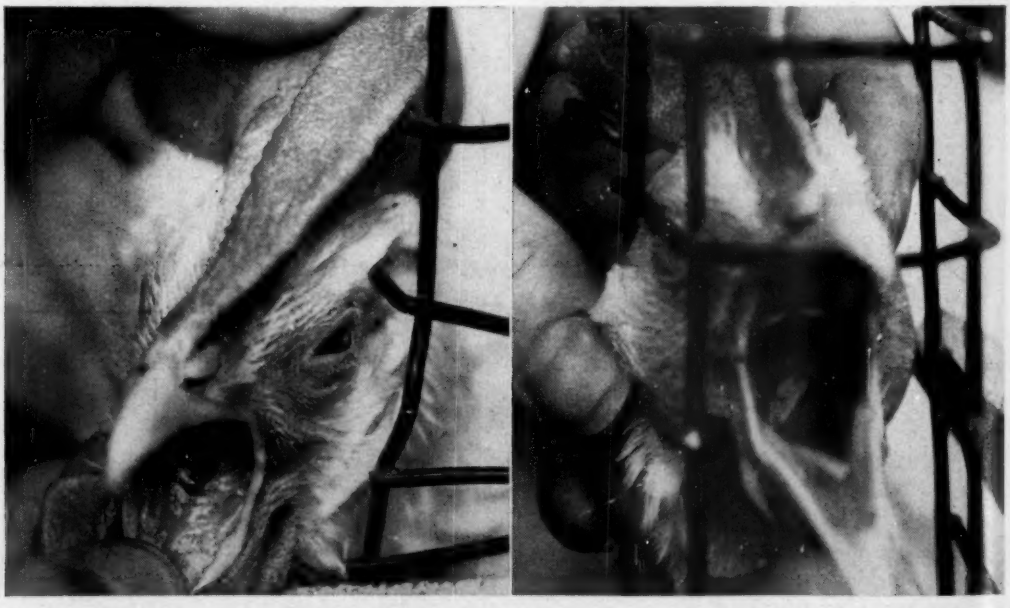

Fig. 1 (left). Early lesion occurring in the oral cavity. Fig. 2 (right). Lesion of longer duration showing the caseated lesion on the surface.

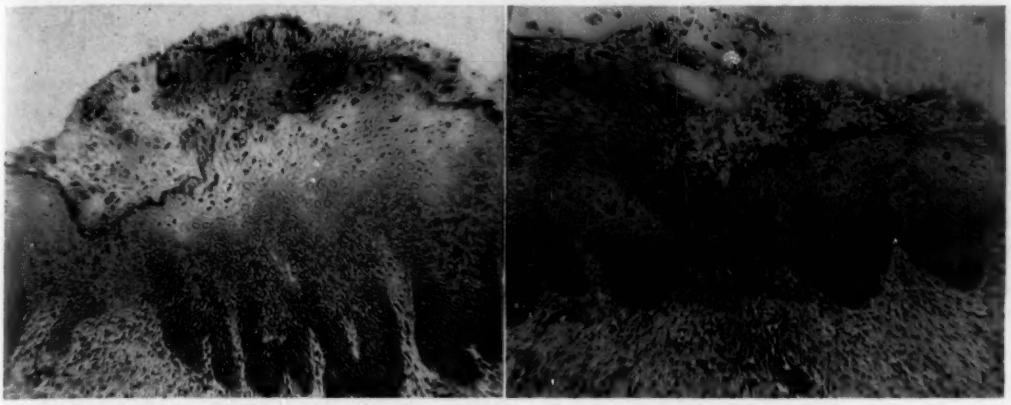

Fig. 3 (left). Section of upper esophagus with an early lesion. Predominating cell type is the polymorphonuclear leucocyte (x 130). Fig. 4 (right). Section from lesion illustrated in figure 2, showing a deeper lesion with a break in the epithelium (x 130).

belonged to the nonpathogenic group. Staphylococci were isolated from eight, yeasts from three, moulds from two and various micrococci from ten of the fifteen cases. No organisms of the Pasteurella or Salmonella genera were isolated.

In 17 out of the 25 cases included in the histological study, a monilia-like organism was observed in the lesions. However, the organisms isolated, the clinical picture, and the pathology of the lesions, did not indicate an infection of monilia.

Trichomonas gallinae was not observed in any of the cases.

SUMMARY AND CONCLUSIONS

Twenty-five cases with the above lesions were studied in gross and microscopically in an effort to determine the relationship, if any, of this condition to the nutritive status of the fowl.

The incidence of the condition was not significantly different between groups of hens on diets containing low and high levels of vitamin A or low and high levels of riboflavin.

All cases were studied histologically. No evidence of the characteristic lesions described in avitaminosis A was found. In contrast, the lesions studied indicated an acute condition in which there occurred little or no permanent damage to the epithelium and mucous glands of the esophagus. Important differences were thus pointed out between these two conditions, which in gross, may appear to some as quite similar, but which upon histological examination, are apparently unrelated.

A limited bacteriological study of 15 cases of esophagitis indicated that the organisms found in the lesions were not the etiological agent.

Caution should be exercised in making a diagnosis so that this condition is not confused with vitamin A deficiency.

A Study of the Composition of the Alveolar Air of the Domestic Animals*

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IN 1893 Haldane and Smith[1] performed some experiments to determine whether there was a poisonous principle eliminated in the expired air. Brown-Sequard and d'Arsonval[2] reported experiments in which they claimed to have shown that such a poisonous principle is expired. The work of Haldane and Smith made it necessary that the composition of the expired air be determined.

This work led to the development of equipment for the determination of the carbon dioxide, oxygen, and nitrogen content of the expired air. Since this work, numerous improvements have been made on the equipment and methods but they still are based on the principles established by Haldane and his coworkers.

It was shown by Henderson, Chillingworth and Whitney[3] that in the air passages the air is not pushed out as a column ahead of the alveolar air in the act of expiration. Some of the air along the borders of the respiratory passages moves out more slowly. Before pure alveolar air leaves the nostrils the air passages must be "washed out" by three or four times the volume of the dead space. The dead space extends from the nostrils to the alveoli.

The collection of an alveolar sample in man depends upon obtaining a sample at the end of a normal expiration by forced expiration. A second sample is obtained at the end of a normal inspiration by a forced expiration. Haldane[4] states that an average of the composition of these two samples is taken as the composition of the alveolar air. This composition will be found constant for individuals under similar conditions.

It was shown by Paul Bert[5] that the physiological action of carbon dioxide, oxygen, and other gases present in the air breathed depends upon their respective partial pressures. In one of the samples tested, the carbon dioxide volume was 5.37 per cent and the oxygen volume was 14.63 per cent. Knowing the temperature and barometric pressure, the partial pressure of each gas in the lungs may be determined. With a barometric pressure of 757.20 mm. Hg, it is known that a certain amount of that pressure is due to water vapor. The amount of water vapor depends on the temperature. Haldane,[6] among others has shown that the air leaving the lungs is completely water saturated. The pressure by the water vapor is 22.152 mm. Hg, at 24° C. The barometric pressure minus the aqueous vapor pressure is 732.15 mm. Hg. The amount of carbon dioxide making up this pressure is 5.37 per cent. Therefore the partial pressure of CO_2 is:

$$\frac{5.37 \times 757.2 - 22.152}{100} = 39.316$$

mm. Hg, or 5.17 per cent of an atmosphere, since 39.316 is 5.17 per cent of standard pressure.

PROBLEM

The study of the composition of the alveolar air of the domestic animals was of

*From the School of Veterinary Medicine, A. & M. College of Texas. Presented before the Section on Research at the 76th annual meeting of the AVMA, Memphis, Tenn., August 28 to September 1, 1939.

[1]Haldane, J. S., and Smith, J. L.: J. Path. Bact., I (1893), p. 168.

[2]Brown-Sequard, C. E., d'Arsonval, A., and Haldane, J. S.: Respiration (1935), p. 16.

[3]Henderson, Y., Chillingworth, F. P., and Whitney, J. L.: Amer. J. of Physiol., xxxviii (1915), p. 1.

[4]Haldane, J. S.: Respiration (1935), p. 20.

[5]Bert, P. Referred to by Haldane, J. S.: Respiration (1935), pp. 31-32.

[6]Haldane, J. S.: Respiration (1935), p. 32.

interest since very little work has been done on this problem. The determination of the alveolar air composition would be an indication as to whether the same principles of transfer of gases from the alveoli to the blood is true of animals as in man.

The determination of the composition of expired air of the domestic animals depends on causing the animal to forcefully expire by physical means. With some modifications the collection of the air sample in domestic animals may be accomplished similar to that for man. The determination of the actual composition is accomplished by the use of a modified Haldane-Henderson gas—analysis apparatus. The nitrogen and other inert gases are determined by difference. Thus, where oxygen equals 15 per cent of alveolar air, carbon dioxide equals 5 per cent. Nitrogen and inert gases make up 80 per cent of the atmospheric air. Best and Taylor[7] state that nitrogen is of no importance since it is inert. A small but constant amount of nitrogen is taken up and dissolved in the plasma, but it is neither retained nor produced within the body and the quantity in the arterial blood is the same.

METHOD OF COLLECTING THE ALVEOLAR SAMPLES FROM HORSES AND CATTLE

In the collection of air samples in domestic animals for the determination of its composition two methods may be used. The first method is to place a tightly fitting mask over the animal's muzzle. The open end is tapered to fit on the collecting tube. The second method is the insertion of a tracheal cannula by which the air is taken directly from the trachea to the collecting apparatus.

In this series of determinations the mask method was used. A rubber tube of sufficient size was employed. This tube must fit securely to prevent leakage around the muzzle. The use of masks for such work has been practiced by such workers as Zuntz and Geppert,[8] and Barcroft, Boycott, Dunn and Peters.[9]

The sampling tube was made of 2-in. copper tubing. This diameter was chosen to prevent any difficulty in breathing since Haldane[10] showed that dyspnea accompanies a rise in CO_2 content of the alveolar air. A short side arm was soldered into the large tube near each end. See Fig. 1, M and N. A pressure bottle filled with liquid petrolatum was clamped upon a small platform. This pressure bottle was connected to the lower tube by means of a length of good rubber tubing. A section of rubber tubing which could be closed by means of a clamp was placed on the upper tube. A small section of glass tubing was inserted for attaching the syringe. The lower end of the tube was equipped with a section of rubber tubing slightly smaller in diameter than the brass tubing.

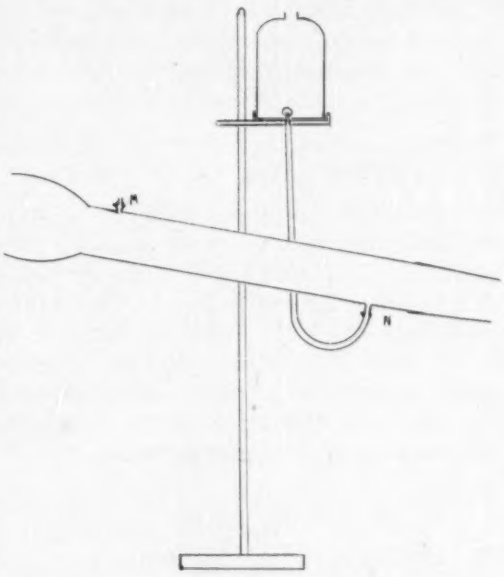

Fig. 1.

The samples were obtained by the use

[7]Best, C. H., and Taylor, N. B.: Physiological Basis of Medical Practice (1937), p. 507.

[8]Zuntz, N., and Geppert, J. Referred to by Mangold, Ernest.: Handbunch der Ernahrung und des Stoffwechsels der Landwirtshaftlichen Nutztiere (1931), p. 399.

[9]Barcroft, J., Boycott, A. E., Dunn, J. S., and Peters, R. A.: Observation on respiration and circulation in the goat. Quart. J. Med., xiii (1919), p. 35.

[10]Haldane, J. S.: Respiration (1935), p. 40.

of the above described apparatus. Dukes[11] explains the use of a mask in which it was placed on the animal's muzzle and breathing continued through the tube. At the end of an expiratory movement, a forceful expiration was caused and the last of the air expired was trapped in the collecting apparatus.

A large clamp was applied across the upper tube and one across the lower outlet tube; the clamps on the side arms were previously closed to avoid contamination.

METHOD OF COLLECTING THE ALVEOLAR SAMPLES FROM GOATS, SHEEP, AND DOGS

A similar equipment with certain changes was used in these species. The collecting tube used was the water jacket of a 300 mm. Liebig condenser. This equipment was used in the same manner as shown above. With the side tubes clamped off, the mask was placed over the animal's nose, forceful expiration produced and the sample trapped in the apparatus.

METHOD OF TRANSFERRING THE AIR SAMPLE FROM THE COLLECTING APPARATUS TO THE GAS ANALYSIS EQUIPMENT

The method described by Haldane[12] includes the use of a vacuous sampling tube. A modified method was used. A 50-cc. Luer syringe with the plunger coated with liquid petrolatum was substituted. Macleod[13] recommends petrolatum to prevent any leakage between the plunger and the syringe. A rubber tube, clamped with a Hoffman tube clamp, was placed on the open end of the syringe. The syringe, by means of the rubber tube, was connected to the upper-side delivery tube of the air collecting apparatus. The clamps, both on the tube from the petrolatum bottle and those to the syringe, were opened. The pressure of the petrolatum caused the air to be forced into the syringe. About 20 cc. were drawn into the syringe. The clamp on the syringe tube was closed. The connection of the syringe and its tube were partially broken and the air was forced out. This was to remove or "wash out" any air in the syringe or side arm which was not "sample air." To further insure the purity of the sample this process was repeated. Fifty cc. were then drawn into the syringe and the syringe tube was clamped off. The syringe and attached tube were removed from the collecting apparatus and placed in the metal clips provided on the gas analysis apparatus.

THE METHOD OF ALVEOLAR AIR ANALYSIS

The modified Haldane-Henderson gas analysis apparatus, shown in fig. 2, was used for the analysis of the atmospheric and respiratory air.

The gas burette was 600 mm. long and had a capacity of 10 cc. From 0 to 7 cc. it was graduated in 1-cc. divisions; from 7 to 10 cc. in .01-cc divisions. Attached to the upper end of the gas analysis burette was a four-way stopcock which is the distinguishing feature of Henderson's modification of Haldane's apparatus. Attached directly to the gas burette by means of glass capillaries were the absorption pipettes for the CO_2 and O_2. Three-way T-bore stopcocks were located above the absorption pipettes to facilitate the cleaning of the apparatus.

Illuminated, opal glass windows were located behind the capillary tube above both absorption chambers to increase the visibility of the levels of the absorbing fluid in the tubes. A large illuminated opal glass window extended the entire length of the rear of the water jacket.

A large knurled wheel promoted the ease with which the rack and pinion for the leveling bulb could be manipulated. A heavy cast iron base, 10″x8″ held the supporting board for the glass assembly. This gave the apparatus sufficient stability to place it at the edge of a table in such a way that the rubber tubing of the leveling bulb would hang free.

A three-way, T-bore stopcock was placed above the burette to aid in the transfer of

[11]Dukes, H. H.: The Physiology of Domestic Animals (4th ed., Comstock Publishing Co., Ithaca, 1937), p. 199.
[12]Haldane, J. S.: Respiration (1935), p. 19.
[13]Macelod, J. J. R.: Physiology in Modern Medicine (1936), p. 355.

the gas from the syringe to the burette. Its use will be described.

The technic of air analysis was similar to the one used in the physiology department of the University of Michigan Medical School.[14]

The stopcocks were properly coated with stopcock grease and introduced. The stopcock grease was applied in sufficient amounts to prevent air streaks but not enough to cause plugging of the passageways.

Stopcock A was turned so that the carbon dioxide absorption chamber B would be open to atmospheric air. Stopcock C was turned so that the control-air chamber D would be open to atmospheric pressure. Stopcock E was adjusted likewise in the oxygen absorption chamber F. The stopcock G of the gas burette was adjusted to permit the burette to be open to room air. The stopcock H was turned so that the gas burette would be open. The levels of the menisci in the pipettes were read and recorded.

To insure the complete saturation of the air sample, enough 1 per cent sulfuric acid was introduced into the gas burette to wet the walls. In order that the air in the control air chambers contained the same percentage of moisture a few cubic centimeters of acid were introduced. The reason for using 1 per cent sulfuric acid was to prevent absorption of the CO_2, which would occur if water alone were used. The water jacket outside the burette and control chamber insured a uniform temperature in each. The water within was agitated at the beginning of analysis of each sample.

Stopcock A was turned 90 degrees clockwise; stopcock C was turned 180 degrees counterclockwise. The mercury bulb was raised to force the mercury up in the gas burette and expel the air present. A 10-cc. sample of room air was drawn in by lowering the mercury bulb. Stopcock G was quickly turned 90 degrees clockwise. If the air in the burette was at room pressure, the meniscus in the CO_2 absorption chamber would come to rest at the original reading. If the meniscus rose, the mercury bulb was raised to return the meniscus in the CO_2 pipette to its original level. The initial volume of the air sample was read to the nearest 0.001 cc. If

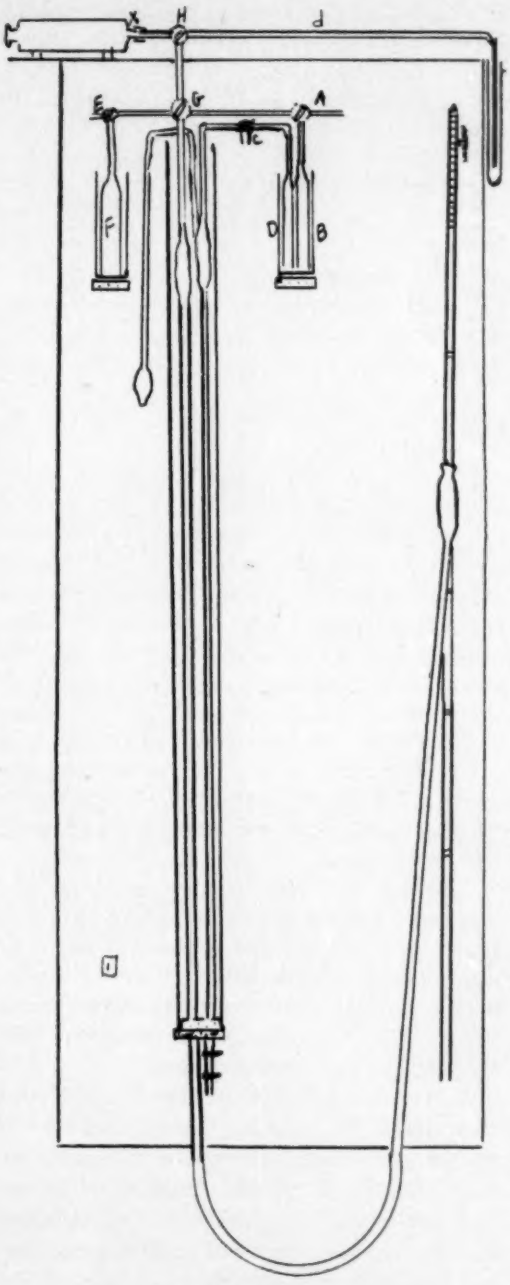

Fig. 2.

[14] Physiology Laboratory Manual of the University of Michigan Medical School (1935), pp. 80-85.

Composition of the alveolar air in five species of animals.

SPECIES	CARBON DIOXIDE PER CENT	OXYGEN PER CENT	BAROMETRIC PRESSURE	TEMP. DEG. C.	CORRECTED PRESSURE MERCURY CO_2	PARTIAL IN MM. O_2
Horse	4.93	15.09	75.75	32	35.442	107.422
Cattle	4.75	15.40	75.45	30	34.189	107.425
Sheep	4.59	15.65	75.85	24	33.565	114.418
Goats	5.18	15.04	75.22	22	38.055	110.488
Dogs	5.32	14.99	75.82	24	38.977	108.593

A summary of five determinations on each animal. Except in the horse, three animals of each species were examined. Only one horse was used.

possible, the volume should have been 10 cc. or slightly less.

Stopcock C was turned 180 degrees clockwise. Any deviations in the volume of gas present, due to variations in temperature, could be observed in the control pipette. The gas in the burette was now exposed to the action of the 10 per cent sodium hydroxide in the carbon dioxide absorption chamber. The mercury bulb was removed from the rack and by alternate raising and lowering, the sample was exposed to the action of the sodium hydroxide. This was repeated several times. The mercury bulb was then lowered until the meniscus in the CO_2 pipettes returned to the original position. A reading was made and recorded. The mercury bulb was again raised and lowered several times to remove any remaining CO_2. If the meniscus of the mercury had not varied more than 0.001-0.003 cc., is was accepted that all the CO_2 had been removed.

A temperature contraction or expansion was shown by a change in the volume of gas in the control tube. If it had fallen one division on the scale because of expansion the drop indicated that a similar volume change had occurred in the sample being analyzed. By lowering the level of the meniscus in the CO_2 absorption pipette the same distance as the meniscus in the control tube the change in volume was corrected. A reading of the mercurial meniscus in the gas analysis burette was now made under the corrected condition.

The stopcock G was turned 180 degrees clockwise exposing the sample in the gas burette to the alkaline pyrogallic acid. The mercury bulb was alternately raised and lowered several times, usually 25 to 30. The mercury bulb was lowered to bring the meniscus in the oxygen pipette back to its original position. The new level of the mercury was read and recorded. To remove any oxygen which may have been left in the carbon dioxide pipette, the stopcock G was turned 180 degrees counterclockwise. The mercury bulb was raised and lowered to remove any traces of oxygen. The meniscus in the CO_2 pipette was returned to the corrected atmospheric level.

Stopcock G was now turned 180 degrees clockwise, and the sample in the burette was again exposed to the pyrogallic acid. The meniscus in the oxygen pipette was returned to its corrected level and the volume of gas remaining in the burette was read and recorded. To insure complete removal, this process was repeated and a second reading obtained. The absorption process was again repeated for oxygen and the menisci were again checked. If there had been no variation greater than .001 to .003 cc., all the oxygen had been removed.

This procedure removed all traces of oxygen and carbon dioxide from the pipettes and burette. It was performed at the beginning of each day's work and was known as a "dummy" analysis. Its purpose was to insure that there was no contamination of the sample. It was used also to check the absorbent solutions to insure their ability to remove the gases of the sample.

An alveolar sample was now ready to be

introduced and analyzed. This was done by turning stopcock H so that a portion of the sample might be forced through tube D and washed of any room air present. Stopcock H was quickly turned 90 degrees counterclockwise. Stopcock G was turned 90 degrees clockwise opening the gas burette to the outside. The mercury bulb was raised to force out the nitrogen and rare gases in the burette. The mercury, with the small amount of 1 per cent sulfuric acid on its surface, should have reached stopcock H. Keeping the mercury bulb at this level, stopcock H was quickly turned 180 degrees clockwise. This opened it to the alveolar sample. The tube clamp X was opened and the sample introduced. This was done by lowering the mercury slowly. The syringe plunger was pressed upon to prevent any marked changes in the pressure in the burette column. A total of 12 to 15 cc. of gas was taken into the gas analysis burette. Stopcock H was turned clockwise a few degrees so that no passageways were left open. The tube clamp on the syringe was closed. Stopcock H was turned so that all except the 10 cc. would be forced out. This extra quantity was forced out by raising the mercury bulb to the 10-cc. level. Then stopcock G was turned 90 degrees clockwise.

The carbon dioxide and oxygen were absorbed by the sodium hydroxide and alkaline pyrogallol respectively. The percentage of carbon dioxide could be obtained by subtracting the reading of the mercurial meniscus at the end of CO_2 removal from the first reading of the sample. An example will explain:

9.980 cc. First reading
9.420 cc. Volume after CO_2 removal

.56 cc. Volume of CO_2 present in above sample

Therefore:

$$\frac{.56}{9.980} \times 100 = 5.61\%$$

Similarly for oxygen:

9.420
7.960

1.48

$$\text{or } \frac{1.48}{9.98} \times 100 = 14.84\%$$

DISCUSSION

The following data are the record of the results obtained. The results obtained in the horse were very similar to those of Zuntz and Geppert.[15] Zuntz and Hageman[16] also reported many determinations at rest which were similar. Later Zuntz and Lehman[17] did more work on the composition of the alveolar air when the animal was at rest which verified the previous experiments.

Barcroft *et al*[18] reported finding the composition of expired air in goats to be:

CO_2 2.95% O_2 17.80%

This work was done on expired air collected in a large bag, which did not represent true alveolar air. By forcing out the alveolar air the results reported herein show that the CO_2 per cent was higher and the oxygen per cent was lower than that obtained by the above investigators in their work on "Respiration and Circulation in the Goat."

Increased excitement due to handling would be a possible source of error. Animals not easily excited were used. Excitable ones were handled frequently until that fault was overcome. Another source of error mentioned was the use of absorbent reagents which would not remove all the gases present. To insure their ability to do this the "dummy" analysis was run each day on room air. The composition of

[15]Zuntz, N., and Geppert, J. Referred to by Mangold, E.: Handbunch der Ernahrung und des Stoffwechsels der Landwirtshaftlichen Nutztiere (1931), p. 403.

[16]Zuntz, N., and Hageman, O.: Landwirtshaftliche Jahrbucher iii (1898), p. 27.

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atmospheric air being very constant throughout the world, any deviation in normal percentage was noticed.

Another precaution used to protect the absorbent reagents was to cover them with a layer of liquid petrolatum. This prevented the absorption of the gases from room air, except through the capillary tubes overnight when the stopcocks were removed.

Since results obtained compare favorably with those reported in earlier investigations for the larger species and the same technic was followed for other species, it is thought that the data herein reported will be of value.

The Renal Circulation of Domestic Animals*

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DESCRIPTIONS of the renal circulation of domestic animals are not available in a complete and agreeable form. Many of them show variance as to the larger vessels and some of the important smaller features have been neglected altogether. The understanding of the normal circulatory system of the kidney is of vital importance, not only from an anatomical point of view, but as a decisive factor in determining structural changes in disease.

The primary object of the work is to demonstrate if possible the basic vascular architecture of the kidneys of the various domestic animals and to prepare permanent specimens that may be used in a visual manner to demonstrate those basic vascular pictures. The method of procedure selected was a modification of the Vinylite corrosion technic developed by Narat, Loeb and Narat.[1]

REVIEW OF LITERATURE

A review of the literature in most every instance gives Bowman[2] credit for his outstanding work. In 1842 he stated that, "All the blood of the renal artery with the exception of a small quantity distributed to the capsule, surrounding fat, and the coats of the larger vessels enters the capillary tufts of the malpighian bodies, thence it passes into the capillary plexus surrounding the uriniferous tubes, and finally leaves the organ through the branches of the renal vein." Bowman's statement with the exception of a few minor details still holds good. The work of Gerlack,[3] Ludwig,[4, 5] Kölliker,[6] Grass,[7] and Huber[8] are all primarily in accordance with his viewpoints. Bowman's convictions meet with discord when compared with the original conceptions of Virchow[9] and later by the indorsements of his work by Golubew.[10] Their work pointed out that the medular portion of the renal tissue was supplied with arterial blood not only from vessels which first pass through the glomerulus (*Arteriae rectae suparae*), but that the primary supply came from direct nutrient branches of the renal artery, the so-called (*Arteriae rectae verae*).

The more recent writers, Morrison[11] and Maximow and Bloom,[12] substantiate the work of Bowman in that no nutrient vessels are found within the medulla or when they are found they are of such minute numbers they are of little consequence.

Huber's[7] work differentiates the types of efferent glomerular vessels of the renal cortex; this phase of the work was also confirmed and extended by Morrison.[11]

Narat, Loeb and Narat[1] describe a procedure using a vinyl resin called Vinylite, as an injection mass; this procedure does not state as to the success obtained in demonstrating the anatomy of the specimens injected.

PROCEDURE

Materials for injection should be obtained as fresh as possible, those kept in the refrigerator or left in the body for only a short time tend to disintegrate to a point where the pressure of the injection will rupture the vessels of the organ.

The use of heparin is recommended for the prevention of the formation of fibrin. Blood vascular systems are easily washed free of the blood cellular elements and the serum, but fibrin once formed is never removed by washing. Heparin is administered to the anesthetized animal intravenously, after which a large vessel is severed and the animal allowed to bleed to death. The entire circulatory system has

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been washed and dried with the animal intact. This procedure is necessary where injections of organs with multiple arteries are used, such as the heart, spleen or lungs. Good injections of the gross arterial and venous systems have been obtained of these organs after the use of heparin. Better injections of the kidney are secured after the removal of the organ from the body; this is due to the necessity of considerable kneading and squeezing of the kidney to remove the last bit of blood and water in the drying process.

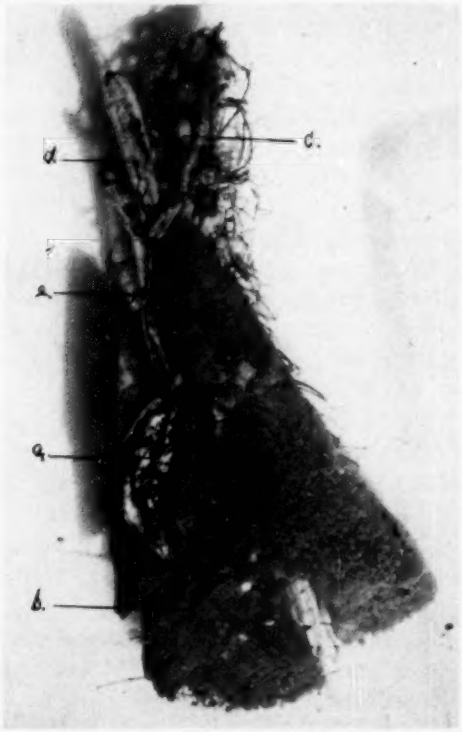

Fig. 1. Ventro-lateral view of thoracic injection of the cat. A—heart, B—lungs, C—spinal cord, D — trachea, E — anterior vena cava (x 2/5).

The organ should be flushed through the artery with tap-water to promote hemolysis; controlled pressure of not over 50 mm. of Hg. should be maintained. It has been necessary to use as much as fifteen gallons of water to completely wash the vessels of the larger kidneys of the horse and ox. The completeness of this process is best determined by a pale gray color of the organ and the absence of any color in the water flowing from the vein and ureter. The drying of the lumen of the vessels is brought about by the insufflation of air. The air is admitted by way of the artery and the pressure is controlled so as not to exceed 150 mm. of Hg. It will be noted that more air will be necessary to maintain this pressure after most of the water has been expelled. Gentle squeezing and kneading will help in the removal of the last quantities of water. Care must be taken in this step to prevent rupturing of the kidney capsule. It is necessary to remove all the water possible from the vessels, as any small quantity remaining will instantly solidify the resin and a dam in the system will result. Even smaller quantities of moisture will sometime cause the cast to

Fig. 2. Ventral view of vinylite cast of the right kidney of a horse (arteries and ureter injected). A—branches of the renal artery entering the cortical areas of the organ, B—perforating capsular arteries, C—ureter (x 2/5) (white spots are air bubbles).

form into a hollow tube and thus produce a brittle specimen. It was thought advisable to rinse the vessels with acetone during the insufflation process to prevent this fault; however, no apparent advantage is obtained if the necessary care and time are taken in the process. The air used must be dry; to insure this a water trap or desiccator or both may be used in the air line. A 12.5 per cent solution of the vinyl resin (Vinylite)* in acetone is used for most injections; however, if the finer structures are most desired, a 10 per cent solution is better.

Coloring agents that may be used are Sudan III, Luxol Fast Blue A. R., Orange

Fig. 3. Vinylite corrosion preparation of the kidney of a dog. A—dorsal subdivision of the renal artery, B—ventral subdivision of the renal artery, C—ureter, D—pelvis.

E, Scarlet R. R., Yellow, Red B, the "Luxol Fast" brand is fast to the acids used. The stains were secured from E. I. DuPont de Nemours and Company. The dyes were added to the acetone solvent to the saturation point, the solution filtered and the resin added to make a 10 or 12½ per cent solution. A saturated solution of the dye is desirable for photographic and microscopic work. Cheap commercial dyes are not suitable as the higher percentage of filler in them tends to make the cast brittle.

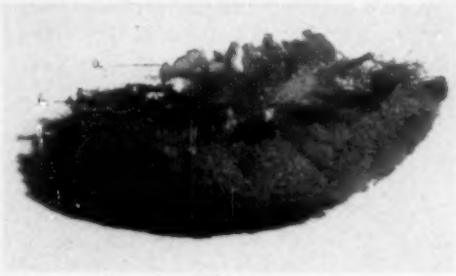

Fig. 4. Inside view of a sectioned right kidney of a dog (arteries and pelvis injected). A—buttress-like evaginations of the renal pelvis, B—collecting tubule casts at the renal crest area (x 2/5).

APPARATUS

The colored solutions of vinylite are transferred to one-gallon pressure bottles. The bottles are equipped with a rubber stopper through which two glass tubes pass, one reaching the bottom of the bottle, the other only through the stopper. The short or air inlet tube is connected by a piece of rubber pressure tubing to the air line; a water trap or desiccator is advisable at this point. A pressure gauge or mercury manometer is connected to the air line at a convenient distance from the pressure bottle. The long glass tube of the pressure bottle is connected to a second piece of pressure tubing fitted at its free end with a renal cannula. All joints must be securely wired to prevent the escape of air or vinylite. The pressures used ranged from 50 to 100 mm. of Hg. for the kidney pelvis, from 100 to 150 mm. for the veins, and from 200 to 600 mm. for the arteries. The higher pressures are necessary for the more complete injections of the glomeruli and capillaries. After the connection of the injecting tube with the vessel has been completed and the pressure built up to the desired point, hemostats are removed first

*Available through Carbide and Carbon Chemical Co., 30 E. 42nd St., New York, N. Y.

from the air tube and then from the injection tube thus allowing the full force of the pressure to hit the vessels suddenly. The organ will distend and the capsule tighten, indicating a complete injection. If multiple injections are desired the process is repeated. In multiple injections of the arteries, veins and pelvis of the kidney it has been found that perfect injections of all three systems is very difficult to obtain;

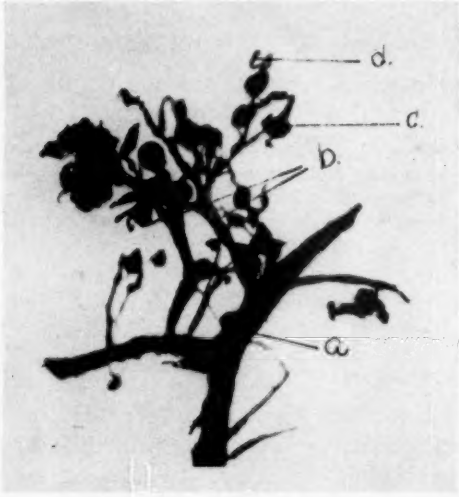

Fig. 5. A—portion of arcuate artery, B—interlobular branches of arcuate artery, C—glomeruli, D—efferent glomerular artery (x 8.8).

for this reason it is advisable to inject the system most desired first and then follow with the others. For example, better glomerular injections are obtained by injecting the renal artery first. Some authors describe a simultaneous method of injection in preparing celluloid corrosion preparations. This procedure may offer some advantages in getting a more uniform dissbursement of the material. However, it has not been tried by the author because of the complicated apparatus necessary.

THE ARTERIAL SUPPLY

It is not uncommon to find two main renal arteries leaving the aorta to supply a kidney. It has been encountered in 10 per cent of the horses and 5 per cent of the dogs examined. These arteries were from ½ to 1½ inches apart at the aorta. Such a development is to be expected as the renal arteries normally divide very promptly after leaving the aorta and will enter the kidney with as high as 14 branches developed. This is more pronounced in the kidney of the horse where most of these arterial branches are distributed in the subcapsular connective tissue on the ventral side of the kidney. They may extend two-thirds the distance from the hilus to the free border of the kidney before penetrating the cortical substance and thence become divided into interlobar arteries. In one instance it was noted that such a perforating artery com-

Fig. 6. Inside view of the sectioned right kidney cast of a horse (arteries and ureter injected). A—renal artery, B—ureter, C—interlobular arteries, D—arcuate arteries, E—interlobular arteries, F—space occupied by a renal lobe (x 2/5).

pleted an anastomotic loop with a main arterial branch entering at the hilus. This is an exceptional case as most of these branches are end-arteries. The renal arteries of the ox, sheep, hog and dog also divide promptly after leaving the aorta,

each of the two main divisions or their branches being distributed to each side of the pelvis in the fatty connective tissue of the hilus. These arteries pursue a course between the pyramids of the kidney in an orderly fashion and are known as the interlobar arteries. In the dog, cat and sheep these arteries are almost completely incapsulated by buttress-like evaginations of the renal pelvis; 14 to 18 of these structures are the most common numbers counted. A few nutrient branches are given off from the interlobar arteries to the surrounding fat and coats of the larger vessels. At the junction of the cortex and medulla the interlobar arteries turn to follow a more horizontal course and form arterial arches across the bases of the pyramids. These vessels are called the arcuate arteries. They in turn give off numerous secondary and tertiary arcuate divisions. The

Fig. 7. Vinylite cast of the termination of an arcuate artery of the kidney of a cow. A—arcuate artery, B — glomeruli, C — efferent glomerular capillaries (x 8.8).

lumen of the arcuate arteries diminishes rapidly. They terminate by suddenly turning their course toward the capsule and branching profusely into interlobular and glomerular vessels. The interlobular arteries arise mainly from the convex side of the arcuate vessels and run parallel with each other toward the capsule. In some animals such as the ox where the fetal lobulation is maintained these vessels run in a radiating fashion toward the capsular surface of each lobe. The infolding at the junctions of the lobes produces an interdigitating effect of the adjacent interlobular arteries. The space occupied by the lobular circulation in such kidneys is quite spherical as has been demonstrated by spaces left in arterial casts by complete blocks of the interlobar arteries. The cortex of the kidney of the horse suggests the arrangement as found in the ox. However, the renal pyramids are completely fused at the crest and the surface of the kidney is smooth. In well-injected kidneys an average of 48 incompletely fused pyramids

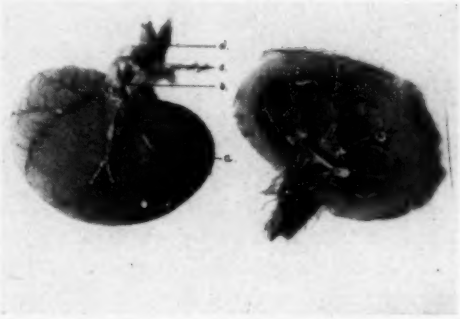

Fig. 8. Vinylite corrosion preparation of a lion's kidney (arteries, veins and pelvis incompletely injected). A—note elaboration of the superficial venous system, B—renal vein, C—ureter, D—renal artery (x 2/5).

have been counted. Fetal lobulation is more distinct in the kidney of the hog. The medullary substance forms a renal crest enclosed within a renal pelvis which is supplied with prolonged outward pockets simulating calyces into which isolated groups of collecting tubules empty. The surface of the hog kidney is smooth. The kidneys of the sheep, dog and cat leave no evidence of fetal lobulation. The pyramids are fused into a continuous renal crest, the surfaces are smooth and no trace of lobulation is apparent in the outer medulla.

The interlobular arteries of these animals run uniformly parallel to each other and at right angles to the surface of the organ. Casts of these arteries from the sheep and hog appear to be finer and more numerous than those of the rest of the domestic animals.

It is not uncommon to find interlobular arteries branching into a number of subdivisions before giving off other branches.

Sometimes the main trunk of an interlobular artery will continue through the capsule of the organ and ramify in the pericapsular fat. This has been observed quite often in the kidney of the ox and horse. Smaller branches may terminate directly into nutrient tubular or capsular capillaries. The greater quantity of the blood of the interlobular arteries is distributed to the glomeruli either directly by way of an afferent glomerular vessel or by way of trunks which terminate by subdividing into two, three or more afferent glomerular vessels.

Good glomerular injections have been prepared with the injection of vinylite; however, not all specimens give uniformly complete results. Yet, however, a sufficient number of specimens of each of the species of the domestic animals have been prepared to study comparatively their structures.

Terminal portions of arcuate arteries breaking up into interlobular branches and bearing clusters of glomeruli have been dissected and mounted dry under watch glasses for classroom study. These mounts are easy to prepare as no preserving fluid is necessary and no difficult air-tight seal need be maintained. The stereoscopic picture obtained by study of these specimens under a binocular microscope surpasses any word picture obtainable from the finest textbooks.

Efferent glomerular vessels and their capillary ramifications are observed in the better injected specimens. By separate venous injections and by carrying out multi-colored injections in the same specimen it is easy to study comparatively the venous systems of the kidneys of domestic animals. The greater bulk of venous blood is drained from the capillaries surrounding the renal tubules of the cortex and is conveyed to the interlobular veins situated between the renal lobules of that region. The interlobular veins flank the arteries of the same name and converge to form arcuate veins and later interlobar veins. In the superficial part of the cortex the veins form star-like figures (*Venae stillatae*) by the convergence of several small radicles to form a common trunk. *Venae stillatae* are quite numerous in the capsule of the kidney of the dog. The main common trunk continues as, or empties into an interlobular vein. As first described by A. E. Belt, the feline family has a superficial venous system in addition to the one described above. Some of the veins draining the capillary plexuses around the renal tubules empty into interlobular veins which do not deliver their blood to the arcuate veins, but reverse the flow toward the capsule, at which place they convene to form a network of finely branched and anastomosing vessels. The final exit is usually by way of four large venous trunks which join first with each other and then enter the renal vein from either side of the kidney at the hilus.

The blood of the renal crest region or pyramids and collecting tubules is conveyed by the *venae rectae* to the under or concave side of the arcuate veins.

CONCLUSIONS

Gratifying results were obtained in the preparation of over one hundred specimens by the method described. The kidneys injected were as follows: horses, 20; oxen, 10; sheep, 5; hogs, 4; dogs, 20; cats, 10; lions, 6; leopards, 2; pumas, 2; elands, 2; and elephants, 1. Other vinylite casts have been prepared from the following structures: The heart and lungs with their surrounding thoracic vessels of the dog and cat; the head, liver and digit of the cat; the liver of the dog; the lungs and air sacs of the chicken; the spleen of the sheep; the heart of the puma, and the digit of the horse. In comparing these specimens with those of other methods the following advantages are offered:

1) Permanent casts resistant to handling can be secured for teaching purposes.

2) Individual vascular trees of casts can be traced to their smaller ramifications.

3) Structural differentiation of the systems of an individual kidney can be demonstrated by multi-colored injections.

4) Structural differentiation of various species can be illustrated.

5) In comparing the celluloid injection casts with those of vinylite it was found that the latter were more resistant to handling, shrinkage when hardened was much less, and the procedure as a whole was much easier and quicker.

6) Vinylite is inexpensive.

7) The acid-fast dyes used reduce bleaching of the specimen in the corroding fluid to a minimum.

8) The specimens may be kept indefinitely in the dry state.

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Editorial

The American Journal of Veterinary Research is dedicated to all veterinarians, not only to the few its name implies. The object is the prompt publication of deserving articles on research and investigation by American veterinarians and their coworkers in the field of veterinary science.

Heretofore, many well-written articles carefully illustrated and studiously tabulated were set aside too long to serve their purpose to the best advantage or rejected owing to lack of space in the JOURNAL. The sequel has been unfortunate. Priority for the authors was jeopardized and prestige belonging to the profession lost through the 40 or more years since research became a dominant factor in the advancement of medical science.

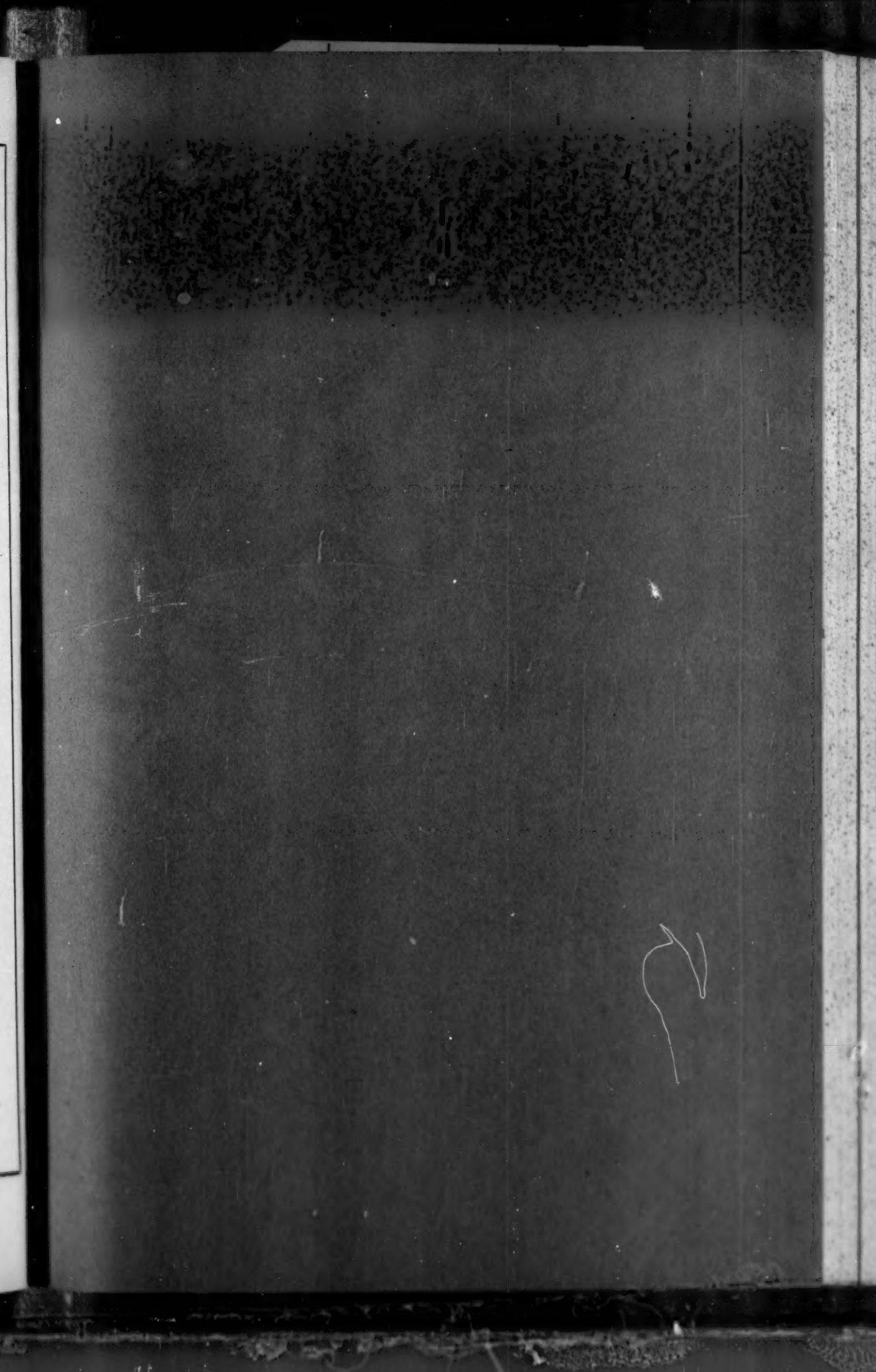
Realizing these disadvantages, the editorial staff recommended to the Executive Board and House of Representatives that the new journal be published without delay. As the recommendation was approved at the 77th annual meeting, August 26-30, 1940, the task was immediately undertaken. Accumulated articles previously accepted for publication comprise most of the contents of the first number.

Portraying the worthiness of veterinary medicine throughout the world is another objective. The editorial staff is, however, keenly aware that competent associates and consultants will be required to keep the new journal in the upper brackets of scientific literature. The names of the aides chosen will be announced in future issues.

The American Journal of Veterinary Research is the result of longtime planning in the inner councils of the Association. It is, therefore, not a medium established merely for the publication of surplus technical material. It should gradually transmute the regular JOURNAL into a more useful monthly for members interested in clinical work and yet concurrently provide them with a means of keeping abreast with current advancements in veterinary science at the nominal cost of $2.50 added voluntarily to the usual annual dues.

The task is undertaken with full realization of the responsibility assumed and of the importance of eliciting the assistance of watchful experts in the various branches of science which the publication is destined to cover in the sequence of coming numbers. The ramifications of veterinary science are too intricate and too numerous for the ken of the average mind.

Future issues will catalogue the contents of American, Canadian and British veterinary journals and, as editorial capacity develops, important material they contain will be abstracted.

AMERICAN VETERINARY MEDICAL ASSOCIATION,

L. A. MERILLAT, Executive Secretary.